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ATTACHMENT C-15

Donaldson (1987)

NeuroToxicology 8 (3):451-462 (1987)

The Physiopathologic Significance of Manganese in Brain: Its Relation to Schizophrenia and Neurodegenerative Disorders

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INTRODUCTION

It was the masklike features of workers, employed in grinding manganese (Mn) ores, especially braunite (a mixture of Mn_2O_3 and $MnSiO_3$), that drew James Couper's attention in Glasgow (Couper, 1837a, b) in the early part of the last century to the possibility that Mn could be implicated as a causative agent in the development of a bizarre disorder with neurological as well as behavioral components. The association between brownstone millers and extrapyramidal movements was recorded in a number of European countries, but was particularly marked in Germany. In a pioneering study, Von Oettingen (1935) studied 70 cases of this syndrome and noted that it was very common in workers exposed to Mn dust. Muscular stiffness, typified by a stacatto-like gait or retropulsion and rigid gaze were prominent features. Hepatic dysfunction was also indicated in some cases suggesting a similarity to hepatolenticular degeneration (Wilson's disease). Micrography, and an intention tremor of the hands were particularly distinctive characteristics found by the early researchers. A distinct psychic manifestation was also evident in the presence of involuntary laughing and auditory hallucinations among the workers.

In a classic review, George Cotzias examined several hundred cases of Mn intoxication (Cotzias, 1958). He recognized that the neurological symptoms of intoxication were related exclusively to inhalation of massive amounts of Mn dust or fumes when Mn was present in a particle size less than 5 μm. Only in those workers directly exposed to dust was intoxication observed. These workers were specifically employed in mining Mn deposits or directly involved in processing ores. It is of considerable import that "old" dusts were found to be less toxic than newly-drilled dusts, and also that braunite was suspected to be especially virulent.

It is unfortunate that these early pioneering observations of (a) particle size, (b) selective neurotoxicity of the aerosol route by dust inhalation, and (c) the powerful influence of the oxidation state of the metal ion on toxic expression, were mainly ignored for several decades since this has led to seriously impeding progress in elucidation of the underlying phenomenon of manganism.

LOCURA MANGANICA

Locura manganica is particularly prevalent in the mining villages of Chile; the term is a colloquialism for "manganese madness". Professor John Cawte has coined the particularly apt title of "Mn psychosis" for this peculiarly bizarre phenomenon. In South America, massive deposits of Mn are mined underground and it is in this geographical location where contemporary clinical and neurobehavioral studies have been undertaken, that much of our present knowledge stems.

Acute Mn intoxication is clinically distinct from the chronic effects of the metal ion, and is distinguished in humans by disorientation,

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memory impairment, acute anxiety, compulsive acts and hallucinations. Chronically, there are extrapyramidal symptoms resembling those seen in Parkinsonism as well as in Wilson's disease. A clinical study of Mn miners (Cotzias *et al.*, 1968; Mena *et al.*, 1967) revealed the somewhat paradoxical findings that the turnover of radioactive Mn (^{54}Mn) was actually accelerated in healthy control workers, indicating the presence of an expanded, rapidly exchanging Mn pool, since there was no significant difference in total body turnover of Mn when normal, nonexposed controls were compared to miners with chronic Mn intoxication.

Other investigators (Cook *et al.*, 1974) demonstrated that urinary concentrations of the metal ion were increased and that this was probably indicative of prior exposure to the metal and did not indicate the extent of neurological damage.

Greenhouse (1982) has noted that there are definite differences in the clinical manifestations of manganism between those patients intoxicated by industrial exposure and miners working actively with deposits of Mn underground. No evidence of rigidity or dystonia, nor indeed of Mn psychosis, was evident in factory workers suffering from poisoning. The same investigator also suggests that chelation therapy, particularly penicillamine, is therapeutically useful in the management of manganism.

A correlation between Mn levels in tissue and the presence of the pigmented polymer, melanin, was found by Cotzias *et al.* (1964). This is an interesting observation since, for a long time, Mn has been employed by dog breeders in England to obtain a hair coat of superior color, texture and quality. In both manganism and Parkinsonism, neuromelanin is diminished in the substantia nigra. Neuromelanin is distinctly different from cutaneous melanin in that it is formed by the nonenzymatic oxidation of dopamine (Das *et al.*, 1978).

The affinity of Mn under *in vitro* conditions for melanin prepared from different sources recently has been studied by Lyden *et al.* (1984). Melanin from beef eyes and human hair and synthetic melanin prepared from dopamine was used to study binding affinity constants. Mn was found to have high affinity for all three types, with the highest binding taking place with natural melanin from beef eye. From these results, as well as *in vivo* results in which whole body autoradiography was used, it was concluded that accumulation of Mn in melanin-containing tissues is the result of the unique predisposition of Mn for binding sites on the polymer. It was considered that free carboxyl groups, hydroxyl, or negatively charged semiquinones were likely candidates. Under conditions of chronic exposure to Mn, accumulation of the metal ion in the pigmented dopaminergic neurons of the substantia nigra may result in lesions and movement dysfunction as a result of production of cytotoxic precursors of neuromelanin. It is curious that the potent Parkinson neurotoxin, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) also has a profound affinity for pigmented basal ganglia tissue.

MANGANESE NEUROCHEMISTRY IN ANIMALS MODELS

Although a plethora of animal models for study of the pathophysiology of Mn has been proposed, in actual fact, the majority of attempts to develop a satisfactory laboratory simulation of manganism have yielded little in practical terms which can meet the rigorous demands of both clinician and scientist for a suitable replica of this phenomenon. A major roadblock to this goal has been the extensive utilization by investigators of rodent species. Because rodents do not possess melanin pigment - a polymer which plays a critical role in Mn action on nervous tissue - use of such species, although generating interesting biochemical data, has not provided a definitive and unifying picture of Mn pathophysiology in humans.

A critically important fact in relation to understanding manganism is that the metal ion is uniquely endowed with the capacity to undergo changes in oxidation state (Cotzias, 1958). Rodier (1955) suggested that the versatility of Mn valence states could underly its ability to induce toxicity.

In 1935, Von Oettingen noted that Mn salts at low dose levels were toxic, but that a 100-fold increase in amounts of manganic Mn were necessary to achieve the same effect. The neurologic symptoms of choreathetoid

movements, rigidity and tremor were found following prolonged (18 mon) injections in monkeys (Mella, 1924). This species possesses pigmented dopaminergic neurons in the substantia nigra. A key step to replicate the Mn dust conditions encountered industrially was attempted when French investigators reported extrapyramidal effects following aerosol inhalation of Mn in monkeys (van Bogaert and Dallemagne, 1945). Severe lesions in the pallidus and subthalamic nucleus of monkeys intoxicated with Mn was found by Pentschew *et al.* (1963).

Parkinson's disease is distinguished neurochemically by a depletion of dopamine in the caudate nucleus. About 80 percent cell loss is required before the clinically characteristic features of bradykinesia, rigidity and tremor appear. Because of the similarity of manganism to Parkinsonism, experimental investigations of Mn intoxication have tended to focus on the effects of the metal ion on catecholamines in the corpus striatum of various animal species. Results using primates, because of their pigmented substantia nigra have furnished pertinent data on the intricacies of manganism. Depletion of dopamine in the caudate nucleus of monkeys was demonstrated by Neff and collaborators (Neff *et al.*, 1969) and the important observation that monkeys exposed to Mn for 18 months exhibited severe neuronal degeneration along with neuromelanin loss in the substantia nigra (Gupta *et al.*, 1980) was a significant milestone in bringing the reality of a laboratory model of manganism to fruition.

In rodents, brain dopamine was found to be decreased following Mn loading (Mustafa and Chandra, 1971; Bonilla and Diez-Ewald, 1974). As noted previously, because research on manganism has utilized rodents, a species without pigmented brain nuclei, progress in pinpointing the underlying mechanisms responsible for the metal ion's ability to elicit neurologic dysfunction in humans has been slow. No clear-cut interpretation of this phenomenon has yet emerged. Results of analyses, even within the same experiment, can vary depending on whether samples were obtained during acute, short-term, or chronic administration of Mn. In this regard, chronic loading with $MnCl_2$ was reported by one investigator (Bonilla, 1980) to increase tyrosine hydroxylase activity in rat neostriatum one month after treatment, but enzyme activity was decreased after eight months.

Other investigators (Chandra and Shukla, 1981) found that Mn treatment produced an initial increase in the content of dopamine, norepinephrine and homovanillic acid in the corpus striatum of rats, but that this was followed by a phase midway when the concentrations were normal, and by yet a third phase (one year) when the content of catecholamines and metabolites had declined significantly. Such discrepancies are likely mediated by the physical state of Mn *in vivo*. Studies of the metabolic involvement of Mn in plant physiology indicate that its function as an essential element in plant nutrition is dependent on the metal's ability to undergo several valency changes, a factor which is useful in response to light-dark changes of phototropism. During Mn excess, the rate of oxidation of Mn^{2+} may exceed the rate of reduction of higher valency species of Mn and result in necrosis of leafy plants (Kenten and Mann, 1950).

In humans, the predominant effect of neurotoxic insult by Mn is likely related to disruption of an endogenous regulatory role fulfilled by different oxidation states of Mn (Donaldson and Barbeau, 1985).

CHRONIC MANGANISM

A striking clinical similarity between chronic Mn neurointoxication and Parkinson's disease is evident from neuropathological studies conducted in each of these disorders. In both conditions there is a reduction of dopamine in the caudate nucleus and of norepinephrine in the hypothalamus, and there is a similar loss of neuromelanin in the substantia nigra (Bernheimer *et al.*, 1973). In the substantia nigra destruction of dopamine neurons results in depletion of dopamine in the nigrostriatal pathway, and is associated with locomotor dysfunction in both manganism and Parkinsonism. REM sleep is also affected in both conditions and the use of l-dopa, the treatment of choice for Parkinson's disease, is also effective in the treatment of manganism (Mena, 1980).

As stated previously, the substantia nigra in humans, as well as in other primates, is pigmented because of the existence of melanin

within dopaminergic neurons of the zona compacta. Since neuromelanin may be formed nonenzymatically from dopamine by autoxidation (Das *et al.*, 1978) and since Mn, particularly in the trivalent pyrophosphate complex (Archibald and Tyree, 1987), is a powerful oxidant of dopamine, it has been suggested that this dopamine-oxidation enhancing property of Mn may explain its ability to induce a toxic lesion in strategic regions of the CNS (Donaldson and Barbeau, 1985).

Dopamine oxidation byproducts are toxic to neuroblastoma cells in culture and the quinone byproducts are also exquisitely cytotoxic (Graham *et al.*, 1978; Graham 1978; 1984).

MANGANESE PSYCHOSIS

Under conditions of neuropathogenesis such as occurs during acute Mn aerosol intoxication in Chilean Mn miners, a clinical condition termed *locura manganica* or "manganese madness" occurs which presents with symptoms reminiscent of schizophrenia as well as amphetamine-induced psychosis. Interestingly, neuroleptics that are potent in alleviating the primary symptoms of schizophrenia are also good antagonists of CNS dopamine receptors (Iversen and Iversen, 1975). Since, in both schizophrenia and amphetamine-induced psychosis, alterations in central dopaminergic receptors are widely accepted to be intimately involved in the pathophysiology of both of these phenomena, it appears likely that one of the toxic expressions of Mn may arise through a special affinity for dopaminergic receptors. Indeed, on the basis of the similarity of neurochemical parameters found in various phases of experimental Mn neurointoxication, there is good reason to consider that such changes in animals may be the pathophysiological equivalent to some aspects of the psychosis found in human manganism.

Certainly there is evidence that Mn, both under *in vitro* as well as *in vivo* conditions in rats, can alter neurotransmitter receptor binding. For example, both agonist and antagonist binding to dopamine receptors is strongly influenced by Mn (Usdin *et al.*, 1980). Also, Mn given intraperitoneally to rats for 15 days, results in increased binding of spiroperidol to striatal membranes (Seth *et al.*, 1981). Administration of Mn to neonatal rats for 14 days resulted in an increase in cholinergic receptor binding in the striatum, but not in other brain regions (Donaldson and LaBella, 1984). Lipid peroxidation activity was also drastically reduced in the rat brain striatum in these animals, an effect which was believed responsible for the change in receptor binding. Interestingly, a dose-dependent decrease in high-affinity binding of the muscarinic cholinergic receptor following incubation of rat brain tissue with catecholamines in the presence of incremental Mn has been found (Donaldson and LaBella, 1984). These effects may be due to receptor destruction or inactivation through membrane damage mediated by free radicals or cytotoxic quinones arising from powerful *in vitro* effects of the metal ion in catalyzing dopamine autoxidation. Also, Mn is a powerful scavenger of the superoxide radical, $0^{2\cdot}$, required for initiation of lipid hydroperoxide activity, and it is feasible lipohydroperoxides may influence receptor binding of neurotransmitters by alteration of membrane processes (*e.g.*, Na-K-ATPase), due to the ability of Mn to influence neuronal redox homeostasis (Donaldson and Barbeau, 1985).

Binding of serotonin to rat cortical membranes is decreased directly in proportion to the formation of malondialdehyde (Muakkassah-Kelly *et al.*, 1982), and dopamine accumulation in synaptosomes is correlated with the rate of formation of lipid peroxides (Pastuszko *et al.*, 1983). Additionally, Sadee *et al.* (1982) has observed that Mn, among several metals, uniquely enhances and stabilizes opiate receptors in brain membranes *in vitro*. Na-K-ATPase activity is enhanced under conditions that inhibit lipid peroxidation (Adam-Vizi and Sergei, 1982) and it is attractive to suggest that, since Mn is a profound inhibitor of this process, endogenous lipid hydroperoxides may exert a regulatory effect on this critical membrane-transport enzyme and thereby exert control of neurotransmitter uptake/release processes.

Clues to unravel the complexities of Mn neurointoxication, especially its hallucinatory effects as observed during acute intoxication, may emerge from study of the normal endogenous function played by the metal ion. It may, in fact, proceed from several

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mechanisms, all intimately related to regulating vital physiological events via its effects on oxyradical formation (Donaldson and Barbeau, 1985). In view of the apparently close relation of Mn with the dopaminergic receptor, it is highly likely that studies using this metal ion as a probe may uncover the underlying intricacies of neurochemical events involved in the pathogenesis of schizophrenia. This would evoke a rich repository of clues leading to the elucidation of this disorder and the biochemistry of hallucinogenic phenomena and unique metallic psychotoxins such as Mn.

DOPAMINE OXIDATION AS A MECHANISM OF MANGANESE NEUROTOXICITY

Parkinson's disease becomes clinically manifest when greater than 80 percent of the catecholaminergic neurons of the substantia nigra have undergone chromatolysis. In Parkinsonism, the amount of neuromelanin contained within the cell bodies of dopaminergic neurons in this region is lost due to what seems to be a preferential destruction of dopamine neurons and concomitant release of the melanin pigment, a polymer of dopamine-derived quinones. This great reduction in pigment is readily observed macroscopically during autopsy.

Loss of melanin also is evident in manganism, a situation which along with other parameters has been considered previously to indicate that both Mn intoxication and Parkinson's disease share a common locus. However, in addition to degeneration of the substantia nigra, cell bodies in other sites, especially the caudate nucleus, pallidum, putamen and thalamus undergo cell loss, a more typical feature of manganism than Parkinson's disease (Graham, 1984). In this connection, striata-nigral degeneration (SND) and, more recently, amyotrophic lateral sclerosis (ALS) may also be candidates for inclusion in the disorders associated with Mn (Donaldson and Barbeau, 1985). *It thus appears that the neurological sequelae of Mn intoxication may represent part of a category of abnormal involuntary movement disorders rather than a specific neurological entity.*

Because of the neurotoxic association of Mn with neurological entities such as SND and ALS, which like progressive supranuclear palsy (PSP), are "clinical conundrums" in that as yet no adequate therapeutic strategies have been discovered for these disorders. Because the underlying etiology of these disorders has not yet been unravelled, studies of Mn interrelationships with neuronal processes may yield a rich repository of clues leading to elucidation of certain brain disorders of unknown etiology.

HYPOTHESES TO EXPLAIN MANGANESE NEUROTOXICITY

Some of the hypotheses formulated by investigators to explain Mn neurotoxicity include: (1) the purported ability of Mn to enhance the autoxidation of dopamine, a process leading to the production of quinones with marked cytotoxicity (Donaldson and Barbeau, 1985; Graham *et al.*, 1978; Graham, 1984); (2) the Mn-catalyzed production of toxic catecholamines, including 6-hydroxydopamine, along with a decrease in protective thiols (Donaldson and Barbeau, 1985; Donaldson *et al.*, 1982; Heilbronn *et al.*, 1982) and (3) the ability of Mn to elicit the proliferation of free radicals, like superoxide ($0^{2\cdot}$) or the hydroxyl radical ($OH^{\cdot}$) which may be toxic *per se* (Graham *et al.*, 1978; Graham, 1984) or by oxidation of Mn^{2+} to a higher valency species which may attack sensitive reactive groups on neuronal membranes, thus enhancing peroxidation of lipids (Donaldson and Barbeau, 1985).

Recent evidence indicates, however, that Mn^{2+} or Mn^{3+}, unlike Cu and Fe, do not produce $OH^{\cdot}$ by the classic Fenton's or Haber-Weiss reactions (Haliwell, 1984). Although there is no direct $OH^{\cdot}$ scavenging activity *per se*, due to its powerful superoxide ($0^{2\cdot}$) scavening ability, Mn^{2+} can prevent production of $OH^{\cdot}$ arising from Fe^{3+} catalysis (Archibald and Tyree, 1987). These investigators have rigorously examined the oxidation-reduction reactions of Mn in the presence of several catecholamines and their derivatives, concluding that regardless of the state in which Mn enters the brain, whether in the $^{+2}$, $^{+3}$ or $^{+4}$ state, it will undergo spontaneous oxidation and dismutation, peroxidatic activity, or $0^{2\cdot}$-mediated oxidation, ultimately giving rise to the trivalent Mn^{3+} species which can efficiently

oxidatively destroy dopamine and other catechols.

An important property of Mn, in relation to its capacity to induce toxic effects, is its ability to accelerate the oxidation of catecholamines. It is likely that this arises through the escalation of Mn^{2+} to Mn^{3+} species. An important adjunct to this reaction is the presence on dopamine of adjacent 3-4 hydroxyl groups. Trivalent Mn could then oxidize catecholamines by one electron transfer reaction thus generating both semiquinones and orthoquinone.

PROTECTIVE EFFECT OF ZINC ON MANGANESE-INDUCED DOPAMINE OXIDATION

Since the progressive neuronal injury that would occur due to oxidation products of dopamine, as well as that arising from the products of the partial reduction of oxygen, (not to mention the serious impairment in neuronal defense mechanisms that would also arise due to glutathione depletion), it is unfortunate that only a paucity of data is available concerning responses and specific mechanisms by which the CNS deals with such toxins.

In early studies set up to examine the ability of some biologically-relevant metal ions in promoting catecholamine oxidation, it was found that zinc (Zn) had little effect in enhancing oxidation of several catecholamines (Donaldson *et al.*, 1981). Further studies indicated that Zn ions could actually impede the ability of Mn ions to potentiate dopamine oxidation. The results of these studies are now complete and confirm preliminary investigations that Zn ions possess the ability to significantly decrease the catecholamine oxidation enhancing activity of Mn ions.

As shown in Fig. 1, Mn clearly potentiates the oxidation of dopamine as measured by the

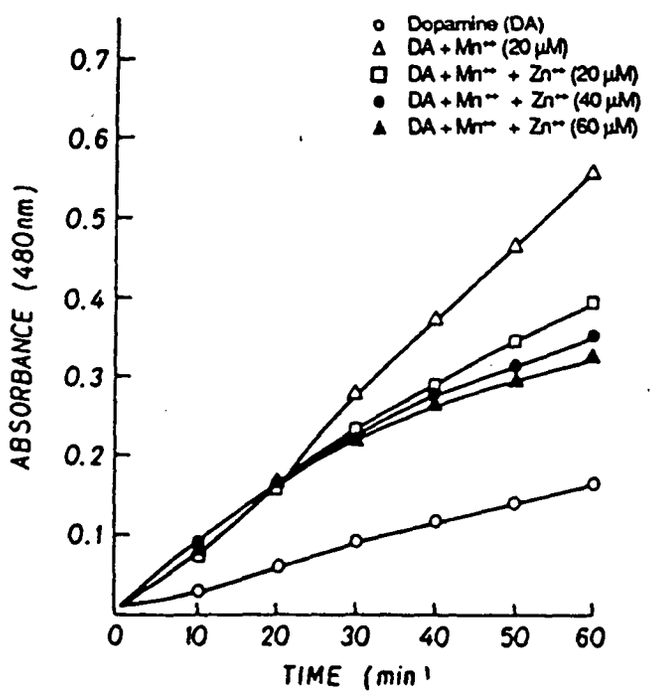
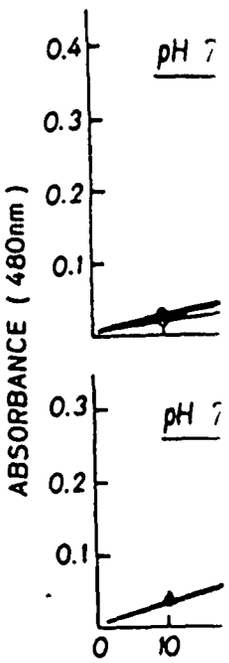


FIG. 1. Effect of Zn^{++} on the manganese-enhanced oxidation of dopamine. Incubations contained 0.05m Tris buffer and dopamine, 2.5×10^{-4} m at 37° C as well as Mn^{++} and Zn^{++} at the concentrations noted. Reactions were initiated by the addition of dopamine to 10 ml beakers incubated in a Dubnoff shaking water bath and terminated by placing the beakers in crushed ice.



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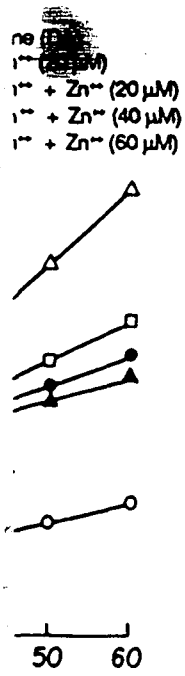

ed oxidation of and dopamine, Zn^{++} at the the addition of water ed

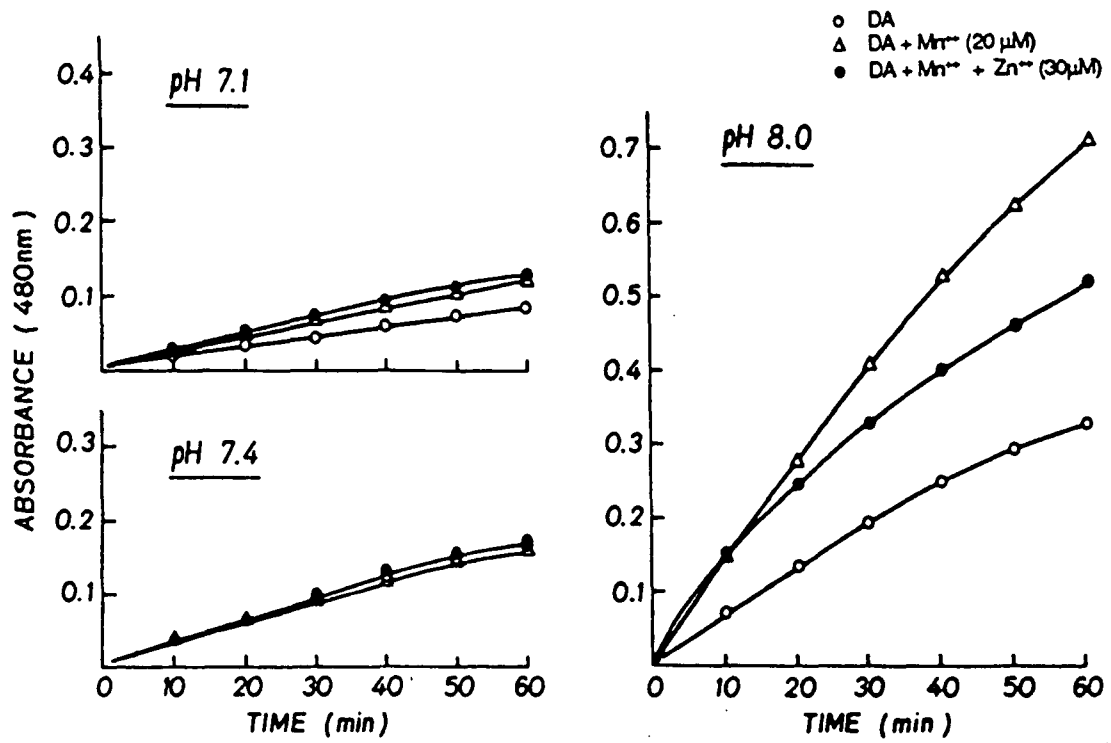

FIG. 2. Effect of pH on Zn^{++} protection of manganese-enhanced oxidation of dopamine.

increase in aminochrome absorbency at 480 nm compared to beakers lacking Mn. However, this potentiating effect is suppressed dose-dependently by the addition of Zn^{++} in concentrations of 20, 40 and 60 μM. Zinc addition to dopamine solutions in tris buffer did not produce an increase in aminochrome formation over that obtained by control solutions containing dopamine only. Fig. 2 indicates that the potentiation of dopamine by Mn is directly related to the pH of the tris buffer. Only slight enhancing activity occurs at near neutral pH values, while basic pH produces marked augmentation of the catecholamine autoxidation rate. Addition of Zn depresses the potentiation due to Mn. Since Zn^{++} ions share similar electronic shell properties to Ca^{++} ions, and since these two metal ions demonstrate mutual antagonistic properties, Ca^{++} salts were included in some experiments, but were ineffective in reducing the Mn-promoted oxidation of dopamine. To clarify the nature of the Zn^{++}-suppressing effects on Mn-enhanced dopamine oxidation, Zn^{++} (30 μM) was incubated with dopamine in an ice bath for one hour and the ability of Zn^{++} to suppress the Mn-promoted dopamine oxidation was then examined (Fig. 3). As noted, previous exposure of dopamine to Zn^{++} did not alter its ability to suppress Mn-enhanced dopamine oxidation.

It is known that Zn has a considerable propensity to form complexes with hydroxyl groups, thus the present investigation indicating protective effects of Zn^{++} on Mn-enhanced dopamine oxidation are probably explained by the affinity of Zn^{++} for hydroxyl moieties. Mn also has a special affinity for the adjacent 3-4 hydroxy group on dopamine and other polyphenols with this arrangement, and in the presence of zinc, this property is probably obtunded due to mutual competitive ion antagonism. Also, vanadium, a powerful oxidant, readily enhances dopamine oxidation, but in the presence of this metal, zinc ions are ineffective in preventing V-induced dopamine oxidation.

In view of the ability of dopamine oxidation byproducts to exert toxic effects against neuroblastoma cells in culture (Graham *et al.*, 1978; Graham, 1984) and to alter cholinergic neurotransmitter binding under

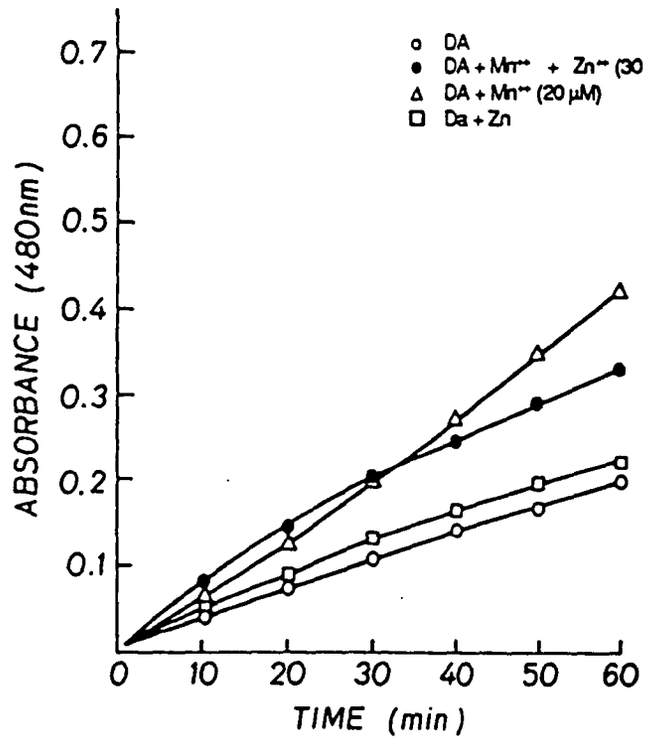

FIG. 3. Effect of time of exposure to Zn^{++} on manganese-enhanced dopamine oxidation.

experimental conditions of Mn excess in the CNS of neonatal rats (Donaldson *et al.*, 1982; Donaldson and LaBella, 1984), it is conceivable that endogenous Zn^{++} in discrete brain compartments may play a protective physiological role related to maintenance of redox balance. Zinc ions are known to play an important stabilizing role in membrane structure and can protect cell biomembranes from disruption by agents which enhance lipid peroxidation, although apparently Zn^{++} itself does not participate in decreasing or attenuating this process (Bettger and O'Dell, 1982).

ECOTOXICOLOGY OF MANGANESE AND THE "GROOTE EYLANDT SYNDROME"

Due to his vision and physical as well as intellectual driving force, Professor John Cawte (Department of Psychiatry, University of New South Wales) focused the attention of the scientific community on a possible relationship between certain peculiar neurobehavioral disorders in remote Northern Australian Territories (particularly among the aboriginal inhabitants of Groote Eylandt in the Gulf of Carpentaria) and the presence of Mn in the environment. The potential importance of these studies on Groote Eylandt lies in the fact that a native population has been exposed to a known Mn-bearing ecology for about 70 years. Although Mn in water and food is relatively nontoxic, aerosol inhalation of this metal ion in dust is associated with neurotoxicity.

In the fetus, as well as in neonates and young children, the CNS is preferentially vulnerable to insult due to a marked ability of the CNS to concentrate Mn. Furthermore, the developing organism has a relatively poorly developed excretory mechanism. In other areas of the world (*e.g.*, Mn in mines in Northern Chile) exposure is intermittent and occurs predominantly among adult miners. In Groote Eylandt, however, the popularion has been exposed through several generations from birth due to the presence of two Mn outcrops on the island, *i.e.*, Emerald River and Angurugu

River. These sites are in close proximity to a large bay on the island near the western coast, named Blue Mud Bay. The blue color is due to the presence of enormous deposits of Mn nodules.

The author (JD) was fortunate to visit Groote Eylandt recently and to have the opportunity to inspect the large Mn ore complex operated by GEMCO, a subsidiary of BHP Australia. The author was also privileged to observe the clinical presentation of some of the aboriginal patients who suffer from a movement disorder referred to by the inhabitants as "bird disease", so termed due to the peculiar strutting leg movements of those afflicted. Although this dysfunction in gait was not relevant to the extrapyramidal movement disorders encountered in Parkinson's disease, they were reminiscent of the phenomenon of *demarche en pied de coq* or "cockwalk" first described by Seelert (1913) and typified by a gait in which the patient uses small steps and in which he tends to rotate the heels, elevating them outward. He progresses without pressing on the flat of his feet, but on the fourth and fifth toes of the metatarso-phalangeal articulations.

Similar movements were reported by the late André Barbeau among ten patients suffering neurological complications of chronic Mn intoxication which he observed during a visit to the Mn mines in Northern Chile (Barbeau, 1984). Also, the "Groote Eylandt syndromes" have medical diagnoses which include motor neurone disorders, in which both upper and lower motor neurone are affected. There is also cerebellar involvement and sometimes dementia.

A complicating feature which precludes clear-cut interpretation of clinical as well as biochemical and environmental factors, and which obscures understanding of this disorder among the islanders, is that petrol sniffing has reached epidemic proportions, particularly among the young.

It is considered significant that Groote Eylandt is located in a similar geographical location (longitude 140°) to a cluster of neurological entities with a high incidence occurring in this region. These are Parkinson-dementia and motor neurone disease which are found in Guam, West New Guinea and the Kii peninsula of Japan. There appears to be a very thin dividing line between the clinico-pathologic features of the neurological entities encountered in this region in distinct contrast to that observed with the same disorders in other parts of the world.

Garruto (1985) notes that Parkinson-dementia occurs in association with a high incidence of ALS, often in the same sibship and even together in the same patient. In Guam, where studies have been carried out by the National Institute of Neurological and Communicative Disorders for several decades, Parkinsonism-dementia is always associated with a progressive dementia, and neuro-pathologically both ALS and Parkinsonism-dementia show the characteristic hallmark of Alzheimer neurofibrillary tangles in brain and spinal cord (Garruto, 1985).

A rational explanation for such diverse conditions may be the presence of a common mechanism of pathogenesis. It is of considerable import that neither offspring of patients, nor of controls, showed a significantly increased risk of developing disease, although risk was higher among spouses of patients. Such factors, considered along with recent epidemiological findings, lead Garruto to conclude "that environmental factors are strong contributors to the etiology of these disorders" (Garruto, 1985). However, no association between ALS and Parkinsonism-dementia and plant and animal toxins, including cycad nut, cassava and fish toxins, on Guam has been noted.

On Groote Eylandt, the amount of calcium in the drinking water is negligible - a factor which may play a crucial role in the pathogenesis of the peculiar neurobehavioral disorders on the island. Studies conducted in the three areas of high foci in the western Pacific, *i.e.*, Guam, Western New Guinea and Kii, have found unusually low concentration of calcium and magnesium in soil and water, an event which was considered of considerable relevance by Japanese investigators who have had a pioneering influence in studying such neurological phenomena (Yase, 1972).

In combination with excessive amounts of metals such as Mn, selenium and aluminum, deficiencies of an essential mineral such as calcium can result in serious impairment of critical absorptive mechanisms, which along with abnormal mineral metabolism, can result in preferential transport to the CNS of neurotoxic metals normally occluded. In the

case of Mn in brain, *it is not necessarily the presence of excessive amounts of ion which can result, but the selective neuronal injury in discrete regions like the basal ganglia and the potential lethality arising from the presence of selectively toxic trivalent species.*

Consequently, a mineral deficiency may be a factor in triggering neurodegenerative disorders due to failure of normal mechanisms controlling cellular permeability and ensuring that redox homeostasis prevails under adverse conditions resulting from mineral imbalance.

MANGANESE AND MPTP-INDUCED HYPERGLYCEMIA IN MICE

Either a deficiency or an excess of Mn in the rat can affect carbohydrate metabolism (Hurley *et al.*, 1984). In this regard, we have confirmed the hyperglycemic effect of Mn chloride s.c. injections in mice, thus extending earlier findings in rats (Hurley *et al.*, 1984). Interestingly, there may be an association between the neurotoxic profile of Mn and its ability to affect carbohydrate homeostasis since alterations in glucose tolerance curves have been reported in patients suffering from chronic manganism (Hassenein *et al.*, 1966). It is possible that monitoring blood glucose may prove a useful index of Mn exposure under occupational conditions, an event which is worthy of further exploration.

Because the potent Parkinson neurotoxin MPTP acts similarly to Mn in depleting striatal dopamine, it was of interest to determine if this agent could also influence carbohydrate metabolism. Results thus far (Donaldson *et al.*, 1987) indicate that MPTP can produce a dose-dependent hyperglycemia in mice which is blocked by the monoamine-oxidase inhibitor, pargyline. Whether both Mn and MPTP exert their hyperglycemic ability by directly affecting pancreatic B-cells or by neuroendocrine mediation is presently being explored.

MANGANESE AFFINITY FOR TOXICITY RELATED TO TISSUE REDOX BIOENERGETIC STATUS

Earlier, it was considered that the expression of Mn toxicity in the basal ganglia of sensitive species was related to the ability of the region to generate excessive amounts of H_2O_2 from elevated oxidase activity. Thus, the neurotoxic profile of Mn expressed by inducing focal lesions in the substantia nigra of humans and primates may be related to the high content of oxidative enzymes in this region. Since divalent Mn salts are oxidized to higher-valency Mn^{3+} in the presence of peroxidase, phenols and H_2O_2 (Kenten and Mann, 1950; 1957) it is conceivable that the substantia nigra may provide the precise neurochemical milieu for Mn to elevate its oxidative status (Mn^{2+} to Mn^{3+}) and thus express cytotoxicity by generating cytotoxic neuromelanin precursors from oxidation of dopamine (Donaldson and Barbeau, 1985).

If this premise is valid, it follows then that tissues with elevated contents of oxidative enzymes (oxidases/H_2O_2) would be selectively sensitive to toxic insult arising from the presence of exogenous Mn. This relationship is indicated in Table 1.

TABLE 1. Relationship Between Susceptibility of Tissues to Manganese Toxicity and Their Redox Bioenergetic Status (Oxidases/H_2O_2).

Tissue	Redox Activity	Toxic Effect
Basal ganglia	Intense	Parkinsonism
Spinal cord		ALS?
Testes	Intense	Impotence, abnormal sperm
Pancreas (B-cells)	Intense	Abnormal glucose metabolism
Macrophages	Intense	Pneumonitis, granulomatous disease?
Leafy plants	Intense	Necrosis

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rkinsonism
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ATTACHMENT C-16

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METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT) IN PETROL: THE TOXICOLOGICAL ISSUES

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ABSTRACT

Methylcyclopentadienyl manganese tricarbonyl (MMT), when used as an octane improver in petrol, leads to increased airborne levels of manganese in the form of Mn_3O_4. The potential health effects of increased airborne manganese are considered in this paper. Manganese, unlike lead which it can replace in petrol, is a normal and essential component of the human diet and the intake from airborne manganese is slight by comparison to the normal dietary intake. The major toxicological effects of manganese, observed after long occupational exposure, are on the lung (manganese pneumonia) and the central nervous system (manganism). The small increase in airborne manganese from the use of MMT in petrol is 3–4 orders of magnitude lower than the level required to produce toxic symptoms of manganese exposure, even in areas of high traffic density, and no health risk from the use of MMT is likely.

INTRODUCTION

Methylcyclopentadienyl manganese tricarbonyl (MMT) can be used as an octane improver in unleaded or leaded petrol. In the internal combustion engine of cars, it is converted into inorganic oxides of manganese, the principle one being manganous manganic oxide ($Mn^{II}Mn_2^{III}O_4$ or Mn_3O_4), which is the major product. Manganese has long been considered a health hazard at the high levels of exposure often found in the occupational environment. This has led to concern regarding its use as a petrol additive, given the comparison with the use of lead in petrol and its associated problems. The health aspects of Mn intake have been considered in previous publications by U.S. EPA (1984), Canadian Health and Welfare (1978) and Cooper (1984). In this paper, the debate regarding the use of MMT is continued and the public health issues of increased atmospheric Mn_3O_4 levels discussed.

EXPOSURE LEVELS

Exposure of humans to MMT, apart from occupational exposure during production, is limited to the inhalation of MMT in urban air or inhalation of vapours of petrol/MMT mix at petrol stations. MMT undergoes rapid decom-

Note: The views expressed in this paper do not necessarily reflect the views of the Australian National Health and Medical Research Council.

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position in sunlight to a mixture of manganese oxides. The half-life for the photolysis of MMT in air and sunlight has been estimated to be ~ 15 s (Ter Harr et al., 1975). MMT levels were measured in Toronto by Coe et al. (1980) and could be detected only in an underground car park at levels of 0.1–0.3 ng m^{-3}. No MMT could be detected in urban air above the detection limit of 0.05 ng m^{-3}.

Exposure to MMT in petrol has been compared to exposure to tetraethyllead (TEL) in petrol (Faggan, 1975). With TEL at 2.5 g Pb gal^{-1} and MMT at 0.125 g Mn gal^{-1}, the partial pressure of MMT in petrol was calculated to be about one-thirtieth that of TEL. Thus, at these levels in petrol, exposure to MMT would be much less than exposure to TEL. While the inhalation toxicity (measured as LC_{50} in rats) for MMT (100 mg m^{-3}) is greater than for TEL (850 mg m^{-3}), the overall hazard should be less.

The use of MMT would be expected to result in increased atmospheric levels of Mn_3O_4, the principal exhaust product. Traces of manganic oxide (Mn_2O_3) may also be present. In the U.S., data from the National Air Surveillance Network estimated the current average urban atmospheric Mn concentration to be 0.03 $\mu g\ m^{-3}$ (U.S. EPA, 1984). A prediction of the likely increase in the average Mn concentration in urban air from the use of MMT can be made by using the ratio of the current lead levels in petrol and air. While not altogether accurate, since the physical properties of the lead and Mn particulates could be different, the figure obtained is probably a reasonable estimate. In Australia, where lead is currently added to petrol at a level of 0.45 g l^{-1}, an average airborne lead concentration of 0.5 $\mu g\ m^{-3}$ was found in Sydney (Roberts et al., 1983). It is clear from the range of values obtained in a number of Australian cities that airborne lead levels can be 10 times this value or higher in areas of high traffic density. Based on the average airborne lead value of 0.5 $\mu g\ m^{-3}$, and a Mn level in petrol of 17 mg l^{-1}, the expected increase in airborne Mn concentration would be 0.02 $\mu g\ m^{-3}$. In areas of high traffic density, therefore, values of 0.2 $\mu g\ m^{-3}$ could be expected. While a level of 0.2 $\mu g\ m^{-3}$ would be a significant increase in Mn levels from the current ambient urban levels of 0.01 $\mu g\ m^{-3}$ found in Australian cities (Goodman, 1976; Roberts et al., 1983), the levels are still low compared with airborne lead levels in urban areas.

MANGANESE AND HUMAN HEALTH

Manganese is the 12th most abundant element and exhibits 11 oxidation states. It has been shown to be essential for growth and reproduction in a number of species, including man, and although the daily requirement is unknown, a diet containing 50 ppm is considered adequate for laboratory animals. The major industrial use for manganese, both alloys and metal, is in steel production. It is also used in the chemical industry and in battery production. The major sources of Mn in the atmosphere are ferroalloy manufacture, iron and steel manufacture and fossil fuel combustion.

Human exposure to manganese can occur by inhalation or by ingestion. Absorption via the lung is highly dependent on particle size, and those larger

than 15 μm are unlikely to reach the thoracic (tracheobronchial and alveolar) region. Particles of 10 μm do not reach the alveoli, but ~35% are deposited tracheobronchially. Alveolar deposition is greatest for particles in the 2–4 μm range and although nearly all particles smaller than 2 μm reach the alveoli, many remain suspended and are exhaled. All particles < 2 μm can be conservatively considered to be deposited in the alveoli (U.S. EPA, 1984). Moore et al. (1975) has estimated the size of Mn_3O_4 particles from combustion of petrol containing MMT to be ~0.26 μm and therefore capable of alveolar deposition. While the rate of lung absorption of inhaled Mn in both animals and humans is unknown, the U.S. Task Group on Metal Accumulation (1973) considered only particles less than a few tenths of a micrometre in diameter to be eventually absorbed into the blood. If all the inhaled Mn_3O_4 were absorbed, even at a point of high traffic density (0.5 $\mu g\,m^{-3}$), the maximum Mn intake would be 10 μg (assuming an adult breathes 20 $m^3\,day^{-1}$). The actual intake would be less than this given than an unknown fraction of the inhaled particles would be exhaled immediately and another unknown fraction would be removed by mucociliary clearance.

The possibility that Mn oxides may be absorbed differently in the lung to other Mn compounds may have been answered partially by the experiments of Mena et al. (1969). In these experiments, an airborne aqueous solution of either 54-$MnCl_2$ or 54-MnO_2 was absorbed (either in the lung, GI tract or both) after inhalation exposure. Excretion rates in the urine were similar for both compounds and the results suggest that 60–70% of the inhaled Mn was eventually swallowed and absorbed via the GI tract.

With regard to oral intake, WHO (1973) has estimated that the daily dietary intake of Mn ranges between 2 and 3 $mg\,day^{-1}$ for adults. Intake for infants is much lower (0.002–0.004 $mg\,kg^{-1}\,day^{-1}$) due to the low concentration of Mn in both breast and cow's milk. U.S. EPA (1984) data on the concentration in drinking water indicates that the level is very low (median level 4 $\mu g\,l^{-1}$) and not a significant source of dietary Mn. Gastrointestinal absorption of Mn in adults is likely to be less than 5% of the total Mn ingested (WHO, 1981). In anaemic subjects, the rate is probably higher, given that the transport mechanism for Mn and Fe are the same (Mena et al., 1974). Clearance from the respiratory tract is an even smaller source of Mn. The U.S. EPA (1984) estimates an average ingestion of 0.00026 $mg\,day^{-1}$ by this route, assuming 100% deposition and clearance at an ambient exposure level of 0.023 $\mu g\,m^{-3}$.

COMPARISONS WITH LEAD

A number of the toxicological concerns regarding increased airborne levels of Mn_3O_4 from the use of MMT appear to have arisen by comparison with the known toxic effects of lead. A major reason why this is an inappropriate analogy is that Mn is already present in the diet and is absorbed via the GI tract at a high level in comparison to the expected level of pulmonary absorption of the Mn oxides. While Mn may be present in a variety of salts and oxides in food

and air, and the relative absorption rates of these forms of Mn may vary, their toxicological effects are considered to be identical following absorption.

Lead, on the other hand, is not a normal or useful component of the diet and its toxic effects are well characterized at low levels of exposure. While blood lead levels are a reasonably good indicator of recent lead exposure, the levels of Mn in blood or urine are an extremely unreliable indicator of recent or long-term exposure due to rapid removal of Mn from the blood stream ($t_{½}$ = 1.5 min). Levels of Mn in blood do not parallel the presence of psychological or neurological symptoms, and display wide individual variation (Roels et al., 1987b). The Mn level in human tissues also has a relatively short half-life (liver, $t_{½}$ = 25 days). No useful comparison, therefore, can be made between blood Mn and Pb levels.

As discussed earlier, comparisons with lead have been useful for estimating expected airborne levels of Mn resulting from the use of MMT. A further comparison might also be useful for determining the likely route of intake of Mn following exposure to Mn_3O_4. Lead-containing particles from car exhausts are absorbed by inhalation or by the ingestion of dust particles on food or other objects in the environment. For children, in particular, the ingestion route is the most significant and the inhalation route is considered a minor exposure pathway. Hence the high blood lead levels found in children living near areas of high traffic density (U.S. DHHS, 1985). Since there is already a high dietary intake of Mn in both children and adults, the contribution from Mn_3O_4-containing dust will not significantly increase Mn intake.

PULMONARY TOXICITY

Inhaled metallic particles can be considered to have one of three fates (Adkins et al., 1980): (i) removal from the lung by exhaled air, by mucociliary mechanisms, by engulfment by pulmonary macrophages, or by lymphatic clearance; (ii) deposited in the lung tissue over a long period with little or no harm; (iii) passage into the systemic circulation. The fate of a particular particle will depend to a large extent on its size, and inhaled Mn particles are expected to be cleared by several of the above mechanisms.

Occupational exposure to Mn dust leading to a high rate of pneumonia has been studied in a large number of epidemiological surveys (see U.S. EPA, 1984). Exposure to high levels of Mn is associated with a syndrome known as 'manganese pneumonia'. The levels required to observe symptoms are generally $> 5\,mg\,m^{-3}$, which is the present limit in the United States for occupational exposure. However, exposure/response relationships are limited by the variable exposure conditions and the number of measurable end points. One study (Nogawa et al., 1973), conducted with Japanese schoolchildren whose school was close to a ferromanganese plant, found there was an increased prevalence of respiratory symptoms (e.g. sputum, wheezing and sore throat) at particularly low exposure levels (3–11 $\mu g\,m^{-3}$). No other study has confirmed these results at such low exposure levels.

Extensive studies in animals suggest that Mn is capable of producing a primary inflammatory reaction in the lung. This condition appears to be exacerbated by secondary bacterial infection leading to bronchopneumonia or pneumonitis and chronic inflammatory effects such as fibrosis. Several studies indicate that exposure to Mn has a depressive effect on the number and phagocytic capacity of alveolar macrophages (see U.S. EPA, 1984). Thus the observed lung changes may be attributed to decreased resistance to respiratory infection.

The majority of studies have been conducted with MnO_2 or $MnCl_2$. Chronic inhalation studies in rats and monkeys using Mn_3O_4 were conducted by Ulrich et al. (1979a,b,c). No significant changes were reported in any of the biochemical or pathological parameters measured, even at the highest dose level (1152 μg Mn m^{-3}). The objective tests of pulmonary function (mechanical and ventilatory properties), however, were not extensive enough to draw any firm conclusions. The possibility of synergistic effects on pneumonitis development as a result of bacterial or viral infection was not assessed. In a similar study in monkeys by Coulston and Griffin (1976), exposure to Mn_3O_4 at a dose level of 100 $\mu g\ m^{-3}$ produced no gross pathological changes and no histopathological changes in the lung. This Mn exposure level was 2–3 orders of magnitude higher than that expected from the use of MMT in petrol.

In an epidemiological study of male workers in a Mn oxide and salt producing plant (Roels et al., 1987a), respiratory tract problems were assessed by a questionnaire as well as by measurement of ventilatory performance. For non-smokers, the frequency of cough and sputum in the cold season and also recent episodes of bronchitis were increased but not to the level of statistical significance. For smokers, the frequency of cough was significantly different to controls. Acute bronchitis was also more prevalent in Mn workers than controls. The difference between Mn workers and controls for acute bronchitis over the last 3 years was particularly striking (38% compared with 19%).

Ventilatory performance of Mn exposed non-smokers was only slightly but significantly different to the performance of controls. No additional changes were observed in Mn exposed smokers. The authors conclude that mild respiratory signs and symptoms may occur in workers exposed to an average airborne Mn concentration of 1 mg m^{-3} over a number of years.

NEUROTOXICITY

The effects of manganese on the CNS in both humans and animals is quite well documented and has been reviewed recently by the U.S. EPA (1984). A brief synopsis is given here, together with a consideration of two recent papers.

Advanced manganese poisoning is described in a syndrome known as 'manganism'. The CNS toxicity can be divided into two stages, the first of which is characterized by psychological disturbances and may be reversible if Mn exposure is terminated. The second stage is a neurological disturbance which is not reversible. The disease begins with anorexia, asthenia and occasional

psychotic behaviour. This can be followed by slurred speech, mask-like face, clumsiness, indifference, spasmodic laughter and crying spells. Severe symptoms are limb rigidity, tremors, excessive salivation and sweating. The symptoms resemble Parkinson's syndrome.

Cases of manganism have been identified in all of those industries associated with the production and use of Mn which produce a high concentration of Mn dusts and fumes. A large number of cases have occurred in Mn mines. Manganese poisoning can result from occupational exposure to Mn dust after only a few months of exposure. The frequency of reporting of the disease is low at exposure levels $< 5\,mg\,m^{-3}$. A few signs of exposure (e.g. tremors at rest) have been reported at exposure levels as low as $0.3\,mg\,m^{-3}$, but this level is still 3–4 orders of magnitude higher than that likely to arise from the use of MMT in petrol.

While a large number of studies on the effects of Mn on the CNS in animals is available, the use of different routes of administration, as well as the measurement of different end points (behavioural, biochemical, histological) has not enabled a clear dose–response relationship to be established. The appropriateness of some animal species for studying Mn toxicity has also been questioned. Rats do not exhibit the wide range of behavioural manifestations described in primates and may not accurately model the neurological disorders observed in man. Exposure of monkeys to $1152\,\mu g\,m^{-3}$ for $24\,h\,day^{-1}$ for 9 months produced no evidence of Mn toxicity (Ulrich et al., 1979a,b,c).

Animal experiments have helped to determine the mechanism of Mn toxicity. Evidence that disturbance of brain neurotransmitter metabolism represents a key effect is accumulating. Chronic exposure leads to a decrease in brain monoamine levels, particularly dopamine (Gianutsos and Murray, 1982). The measurement of brain Mn concentrations following chronic exposure may lead to a better understanding of dose–response relationships in animals. A recent paper by Bird et al. (1984) on the Mn level in monkey brain during a 2-year exposure has addressed this problem. After exposure to levels of $30\,\mu g$ $MnO_2\,m^{-3}$, dopamine levels in both caudate and globus pallidus were depressed while Mn levels in the globus pallidus were increased. No neurological changes were observed during the exposure period and the results suggest that exposure at levels $> 30\,\mu g\,m^{-3}$ or over longer periods may be required to observe such changes. Since, in humans, the first signs of toxicity appear as psychiatric manifestations, this may indicate the involvement of dopamine pathways which project to the frontal lobe. This study strongly suggests that neurological changes cannot be expected in humans until high levels of exposure are reached.

In an epidemiological study of Mn workers in a Mn oxide and salt producing plant by Roels et al. (1987a), subjective symptoms, psychomotor tests, and neurological examinations were recorded. Significant increases in the level of fatigue, timitis, trembling of fingers and irritability were noted. Neurological examination revealed differences in trunk rigidity only. Psychomotor tests revealed alterations in simple reaction time, audioverbal short-term memory

capacity and hand tremor. The authors estimate that a time-weighted average exposure to airborne Mn dust of $\sim 1\,mg\,m^{-3}$ over a number of years may lead to the occurrence of pre-clinical signs of intoxication.

OTHER CHRONIC TOXICITY

Two other possible areas of Mn toxicity have been considered, namely oncogenicity and reproductive effects. Given the relatively low intake of Mn into the bloodstream after airborne Mn_3O_4 exposure, an oncogenicity study by the inhalation route could only be useful for the detection of lung tumours. Since there has been no reported increased incidence of lung tumours among occupationally exposed individuals, often at very high doses (see WHO, 1981), the likelihood of Mn being a lung carcinogen seems small. In a paper by Stenback and Rowland (1979), intratracheal instillation of MnO_2 dust (1.5 mg once a week for 20 weeks) into hamsters did not increase the incidence of lung tumours and nor did it enhance the level of tumours produced by concurrent instillation of benzo[a]pyrene.

Interest in the possible effects of Mn on reproductive parameters in males has centred around reports by manganese workers of impaired sexual behaviour in the form of diminished libido and impotence. Animal experiments have thus concentrated on morphological and biochemical changes in testes. In the experiments of Chandra et al. (1971, 1973) degenerative changes in semeniferous tubules were observed after 150 days of intraperitoneal administration of $MnCl_2$ at a dose of $8\,mg\,kg^{-1}\,day^{-1}$. Other studies have not been in agreement with the Chandra results, and no definite effects of Mn after oral or inhalation exposure can be identified. The only animal study performed with Mn_3O_4 is that by Laskey et al. (1982). Serum testosterone levels and epididymal sperm counts were depressed at dose levels $> 35\,mg\,kg^{-1}$, which are well above the likely human exposure dose level.

In an epidemiological study by Lauwerys et al. (1985), the number of children fathered by workers exposed to a median Mn concentration of $0.97\,mg\,m^{-3}$ was significantly lower than the expected number. This level of exposure is approximately 3–4 orders of magnitude higher than the Mn exposure level likely to arise from the use of MMT.

CONCLUSIONS

Potential toxicological effects of increased airborne Mn appear to be restricted to the pulmonary and central nervous systems. Effects in the lung such as inflammation, pneumonia and bronchitis are clearly evident in both animals and humans exposed to relatively high levels of Mn, however individual susceptibility and toxic manifestations seems to vary greatly, even after very long exposure periods. Further low level chronic exposure experiments in animals may be necessary to define a no-effect level, particularly with regard to ventilatory performance, which appears to be one of the most sensitive indicators

of lung damage. Further studies are also necessary to measure possible changes in susceptibility to respiratory infection after low-level chronic exposure. The data available at present, however, indicate that the increased level of airborne Mn in the form of Mn_3O_4 generated by the combustion of MMT would be approximately three orders of magnitude lower than the level necessary to produce adverse effects in the lung.

Similarly with regard to CNS effects, both animal and human studies to date suggest that relatively high levels of Mn intake are required to produce symptoms of toxicity. In humans, the long time periods required to produce symptoms suggest a cumulative effect. Further research into the mechanism of Mn-initiated CNS effects may lead to a better understanding of dose–response relationships.

On the basis of present information, there is no toxicological evidence to suggest that the increased level of airborne Mn resulting from the combustion of MMT as a petrol additive is likely to constitute a health risk to the general population.

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ATTACHMENT C-17

Cooper (1984)

THE HEALTH IMPLICATIONS OF INCREASED MANGANESE IN THE ENVIRONMENT RESULTING FROM THE COMBUSTION OF FUEL ADDITIVES: A REVIEW OF THE LITERATURE

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Methylcyclopentadienyl manganese tricarbonyl (MMT) is effective in raising the octane level of gasoline and is currently used in Canada for that purpose in a maximal concentration of 18 mg Mn/l (slightly less than 0.07 g Mn/U.S. gal). It has been estimated that if MMT were used in all U.S. gasoline in these amounts, the median increase of Mn in ambient air would be not more than 0.05 μg Mn/m³, with increments generally less than 0.5 μg Mn/m³ along urban corridors. The scientific literature was reviewed to determine how the increases in environmental manganese predicted from MMT use would relate to the amounts in the natural environment and necessary to life and to the concentrations associated with toxic effects.

Even with additional manganese from the use of fuel additives, total Mn intakes would remain within the range of average amounts absorbed from food and water. Respirable manganese in ambient air due to MMT combustion would be many order of magnitude below the concentrations associated with occupational manganism and respiratory problems and also below those reported in isolated episodes of respiratory symptoms in communities near ferromanganese plants.

Evidence was reviewed on the possibilities of: (1) increased absorption of inhaled manganese compared with ingested manganese; (2) hypersusceptibility of infants and persons of advanced age; and (3) increased absorption associated with iron deficiency. While relevant to high levels of exposure, these factors would not be expected to lead to toxic effects from the very low concentrations of Mn resulting from MMT use.

Experimental animals that inhaled the combustion products of MMT in concentrations of approximately 10, 100, and 1000 μg Mn/m³ for 9 mo did not show toxic effects, although there was temporary elevation of tissue levels of Mn. Rhesus monkeys, susceptible to the neurologic effects of Mn, showed no symptoms after inhaling the combustion products of MMT in concentrations of 100 μg Mn/m³ for up to 66 wk. Monkeys exposed to 5000 μg Mn/m³ also showed no symptoms.

There is thus a wide margin of safety between the intakes of Mn essential to health and the high concentrations that have been associated with toxic effects. The small amounts of manganese added to the environment by the combustion of MMT used as a fuel additive would be comparable to the normal background and should not create health problems.

This report was prepared at the request of the Ethyl Corporation, which asked for an objective review of all pertinent data. Thanks are extended to Mr. C. A. Hall and Dr. Gary Ter Haar of that corporation for their assistance in providing reference material and to Barbara Walker and Barbara Speed for their invaluable help in completing the manuscript.

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INTRODUCTION

With continued interest in the use of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline additive, it is timely to review the evidence bearing on the possibility of effects on human health from its combustion products. This review updates a report prepared in June 1977 dealing with the use of MMT in unleaded gasoline in the United States (Cooper, 1977). At that time it was concluded that the slight increase in airborne manganese that would result from widespread use of MMT would be within the range of normal manganese intake and should not have any effect on health.

There have been a number of comprehensive reviews of manganese toxicity in recent years (Schroeder, 1970; National Academy of Sciences, 1973; U.S. Environmental Protection Agency, 1975; Matrone et al., 1977; Stokinger, 1981; World Health Organization, 1981). There will be no attempt to duplicate these reviews. Instead, this report is addressed to several key questions relevant to possible harmful effects of MMT combustion products:

1. How would the increments in manganese intake predicted from MMT use relate quantitatively to normal background levels and to levels known to be toxic?
2. Are there differences in the absorption, distribution, and excretion of *inhaled* manganese, as contrasted with *ingested* manganese, which would make small increases in airborne manganese unusually hazardous?
3. Would individuals with iron-deficiency anemias be unusually susceptible because of increased absorption of Mn?
4. Are infants hypersusceptible, because of increased intestinal absorption and poorly developed blood-brain barriers to metals?
5. Are there effects, other than those on the central nervous system associated with high concentrations of Mn, that deserve consideration? These include acute respiratory disease, interference with hematopoiesis, reproductive problems, mutagenicity, and carcinogenicity.
6. From consideration of all the above factors, is the use of MMT as a fuel additive acceptable in terms of the public's health?

THE USE OF MMT AS A FUEL ADDITIVE

MMT (Fig. 1) in relatively low concentrations has proven to be effective in raising the octane level of gasoline. It is also used in limited quantities as an additive to fuel oils. It is widely used in unleaded gasoline in Canada and on top of lead in some U.S. gasolines.

MMT is a highly toxic compound (Hysell et al., 1974; Stokinger, 1981), and stringent hygenic precautions are essential during its manufacture and handling prior to incorporation in gasoline or fuel oil. However, MMT emitted into the atmosphere is photochemically decomposed, with a very

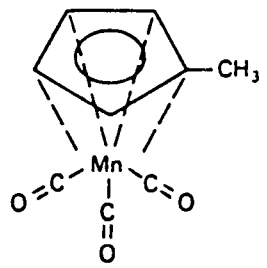

FIGURE 1. Methylcyclopentadienyl manganese tricarbonyl.

brief atmospheric half-time (Ter Haar et al., 1975). At a maximal concentration currently being used in Canada, 18 mg Mn/l or slightly less than 0.07 g Mn/U.S. gal, there is no evidence to indicate that it is a toxic hazard to those who dispense or use fuel.

The combustion of gasoline containing MMT produces manganese oxides—MnO, MnO_2, and Mn_3O_4—with the tetroxide being the principal manganese compound emitted (Ter Haar et al., 1975).

MANGANESE IN THE ENVIRONMENT

In order to place the environmental effects of using MMT in perspective, it is desirable to summarize information on the amounts of manganese present in soil, water, food, and ambient air, as well as the concentrations in the workplace that have been associated with toxic effects.

Soil and water. As manganese is the twelfth most abundant element in the earth's crust, manganese compounds are found in most soils and rock types, the average concentration being about 1000 mg Mn/kg or parts per million (ppm), but with wide variation. Matrone et al. (1977) have summarized the abundance of manganese in rocks and sediments, ranging from 500 to 8500 ppm. Concentrations in soils also vary widely but average values in several series of analyses range from 560 to 850 ppm (National Academy of Sciences, 1973). Concentrations reported in natural water supplies range from less than 1 μg to over 100 μg/l (World Health Organization, 1981). Two U.S. surveys of drinking water reported in 1964 and 1966 showed median levels of 5 and 10 μg/l (World Health Organization, 1981). The first survey showed 97% of samples below 100 μg/l. The 1962 U.S. Public Health Service drinking water standard for manganese sets 0.05 ppm (50 μg/l) as the maximal permissible concentration, based on economic and esthetic reasons (Bull and Craun, 1977).

Food. Schroeder et al. (1966) have documented the manganese content of a number of foodstuffs. These may vary from sample to sample, but whole grains, cereals, nuts, and condiments have high concentrations. Dairy products, meat, poultry, and fish are relatively low. Table 1 illustrates the wide range of Mn found in a number of representative foods. It is of interest,

TABLE 1. Manganese in Foods[a]

Food	Manganese (wet weight, μg/g)	Food	Manganese (wet weight, μg/g)
Whole wheat, seed	11.32	Raisins, package	4.68
Bread, white	1.78	Apple	0.31
Bread, whole wheat	1.43	Orange	0.35
Oatmeal	2.72	Peach	1.02
Corn meal	2.05		
Macaroni, dry	10.56	Pecans	35.09
Grapenuts	30.76	Peanuts, salted	6.91
Milk, whole	0.19	Spinach, fresh	7.77
Milk, dry skimmed	0.00	Beets, fresh	0.41
Butter	0.96	Beans, canned	0.24
Eggs, whole	0.53	Peas, fresh	0.64
		Tomatoes, canned	0.30
Beef, roasting	0.05		
Lamb chops, lean	0.34	Black pepper	47.48
Chicken breast	0.21	Cloves	262.86
		Garlic powder	0.45
Halibut steak	0.12	Coffee, ground	20.65
Scallops, fresh	0.11	Coffee, infusion	0.85
Clams, fresh frozen	0.00	Tea, leaves	273.58
		Tea, infusion	6.9
Cod liver oil	4.95		
Corn oil	1.00		
Safflower oil	0.00		

[a]Selected from Schroeder et al. (1966), Table 6, pp. 551-552.

for example, that a cup of coffee may contain 150 μg Mn, while a cup of tea may contain over 1200 μg.

Ambient air. With the wide distribution of manganese, low levels can be found in most samples of airborne dust, but in rural areas and cities without major industrial activity, the concentrations are usually quite low. The World Health Organization task force (1981) concluded that annual average concentrations above 0.1 $\mu g/m^3$ were invariably man-made. Data summarized by the U.S. Environmental Protection Agency (1975), based on analyses in 600 sites in the United States, half urban and half nonurban, showed annual average Mn concentrations less than 0.1 $\mu g/m^3$ in 80% of sites and 0.3 $\mu g/m^3$ or more in 4.7% of the sites. Urban areas without major iron and steel activity range from 0.03 to 0.07 $\mu g/m^3$. In areas with steel-producing plants, concentrations above 0.5 $\mu g/m^3$ are common, with many maximum 24-h levels above 5.0 $\mu g/m^3$ and quarterly averages above 1.0 $\mu g/m^3$. The highest annual average on record, 8.3 $\mu g/m^3$, was observed in the Kanawha Valley Air Pollution study in West Virginia during 1964 and 1965, (National Academy of Sciences, 1973). Ondov

ood	Manganese (wet weight, μg/g)
package	4.68
	0.31
	0.35
	1.02
	35.09
. salted	6.91
. fresh	7.77
esh	0.41
anned	0.24
sh	0.64
:s, canned	0.30
pper	47.48
	262.86
)wder	0.45
ground	20.65
nfusion	0.85
es	275.58
sion	6.9

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et al. (1982) reported concentrations of Mn in various U.S. cities from 1973 to 1978 ranging from 17 to 170 ng/m³ (i.e., 0.017-0.17 μg/m³).

Ambient air concentrations reported in studies of possible health effects, to be discussed in a later section, cover a wide range of peak and average values. With a few exceptions they are consistent with the concentrations reported by the EPA.

In summary, in the absence of industrial point sources, average airborne manganese levels are usually less than 0.1 μg/m³, a concentration that is a convenient reference point. In many urban areas concentrations between 0.1 and 1.0 μg/m³ can be found, while near ferromanganese operations, concentrations in the range of 1 to 10 μg/m³ have been reported, with occasional peaks above 10 μg/m³.

The predicted increment from the combustion of MMT. By analogy with the airborne concentrations of lead which result from the use of tetraethyl lead, Ter Haar et al. (1975) estimated that if all gasoline contained MMT in a concentration of 0.125 g Mn/U.S. gal (33 mg Mn/l), the median increase in airborne manganese in urban sites in the National Air Sampling Network survey would be 0.05 μg/m³. Along urban street corridors and expressways, the authors estimated that manganese concentrations would generally be less than 1 μg/m³, even under the most unfavorable traffic and weather conditions. The lower concentrations of additive now being recommended or used would result in correspondingly decreased increments of Mn. Thus, the 18 mg Mn/l (0.068 g Mn/U.S. gal) now permitted in Canada would lead to an estimated median increase of about 0.025 μg Mn/m³, with values along expressways generally less than 0.5 μg Mn/m³. Pierson et al. (1978), after measuring Mn concentrations in tunnels of the Pennsylvania turnpike in 1975-1977 during a period of MMT use, concluded that the predictions of Ter Haar appeared to be essentially correct.

Joselow et al. (1978) suggested that the use of MMT in Newark, N.J., had resulted in an increase of manganese in soils near major traffic arteries and caused slight increases of blood manganese in school children. These studies were seriously deficient as circumstantial evidence relating manganese concentrations to the use of MMT, the amount of which could only be inferred. The concentrations found in soil samples ranged from 100 to 600 μg/g, in the lower portion of the range naturally found in soils. The concentration in street dust next to a major roadway (330 μg/g) was only slightly higher than that 70 m distant (290 μg/g). Even if the trivial difference were real, it would be impossible to relate it to MMT use as contrasted with natural variations in soils and the Mn coming from rusted metal and the wearing of manganese alloys stirred up by traffic. The evidence adduced from correlations between manganese and lead concentrations in the blood of children did not take into account the positive association between Pb and Mn levels in blood shown by Zielhuis et al. (1978), which did not appear to reflect relative levels of exposure.

Occupational exposures. After consideration of the concentrations of manganese now present in ambient air and the very small increases that might result from MMT use, it is instructive to review the levels of occupational exposures, including those known to have been associated with toxic effects.

The current workplace standard set by the Occupational Safety and Health Administration (OSHA) (1981) is 5000 $\mu g/m^3$ as a ceiling value (Table 2). The American Conference of Governmental Industrial Hygienists (ACGIH) had adopted the same ceiling value in 1963 and in its documentation (1980) states its belief that this provides an ample margin of safety for manganese and most inorganic compounds. For manganese tetroxide (as of 1978) and manganese fume (as of 1979), however, they have recommended a thershold limit value (TLV) of 1000 μg Mn/m^3 as a time-weighted average (TWA), and for the fume, 3000 $\mu g/m^3$ as a short-term exposure limit (STEL). The World Health Organization Study Group (1980) recommended an occupational limit for manganese in air of 300 $\mu g/m^3$. For a number of years this has been the standard in the U.S.S.R.

As shown in Table 3, there have been many documented occupational exposures that averaged in the 1000 to 10,000 $\mu g/m^3$ range over long periods. Some were in the range of 10,000 to 1,000,000 $\mu g/m^3$. In most of these situations there were workers who exhibited serious toxic effects. There are no documented chronic toxic effects as manifested by central nervous system disease or pulmonary diseases associated with exposures below 5000 $\mu g/m^3$ (ACGIH, 1980). In this context, increases in average exposures of 0.025 to 0.1 μg Mn/m^3 appear trivial.

TABLE 2. Guidelines and Standards for Manganese in the Workplace[a]

Source	Applicable dates	Guideline or standard
ACGIH TLV	1946–1959	6000 $\mu g/m^3$ (TWA)
	1960–1962	5000 $\mu g/m^3$ (TWA)
	1963–	5000 $\mu g/m^3$ (Ceiling value)
	1978–	1000 $\mu g/m^3$ (TWA for Mn tetroxide)
	1979–	1000 $\mu g/m^3$ (TWA for Mn fume)
		3000 $\mu g/m^3$ (STEL for Mn fume)
OSHA	1972–	5000 $\mu g/m^3$ (Ceiling value)
WHO	1980–	300 $\mu g/m^3$ (TWA)

[a]Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; OSHA, Occupational Safety and Health Administration; WHO, World Health Organization; TLV, threshold limit value; TWA, time-weighted average; STEL, short-term exposure level.

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1000 $\mu g/m^3$ (TWA for Mn fume)
3000 $\mu g/m^3$ (STEL for Mn fume)
5000 $\mu g/m^3$ (Ceiling value)
300 $\mu g/m^3$ (TWA)

ental Industrial Hygienists; OSHA, Health Organization; TLV, threshold ure level.

TABLE 3. Examples of Levels of Occupational Exposure to Airborne Manganese[a]

Operation	Range of averages[b] (μg Mn/m^3)	Range of peak levels[b] (μg Mn/m^3)	References
Ore crushing mill	10,400–173,000	–	Flinn et al. (1940)
Ore crushing	62,500–250,000	–	Ansola et al. (1944)
Permanganate manufacture	300–250,000	–	Lloyd Davies (1947)
Mining, mine #1	187,000–926,000	–	Rodier (1955)
Mining, mine #2	65,000–814,000	–	Rodier (1955)
Mining, 1954	500–16,300	–	Schuler et al. (1957)
Mining, 1955	1,800–46,000	–	Schuler et al. (1957)
Ferromanganese production	2,300–4,700[c]	–	Whitlock et al. (1966)
Mn ore processing	5,030–11,100	5,250–31,500	Tanaka and Lieben (1969)
Ferromanganese production	1,600–8,600	4,430–20,130	Tanaka and Lieben (1969)
Dry battery manufacture	6,800–42,200	–	Emara et al. (1971)
Ferromanganese production			
Old preparation plant	27,000–1,122,000	52,000–1,750,000	Smyth et al. (1973)
Blast furnace and pig casting[d]	120–13,300	1,900–206,000	Smyth et al. (1973)
Mn processing	2,100–12,900	5,000–61,500	Smyth et al. (1973)
Ferroalloy production	301–20,440	–	Šarić et al. (1977)
Ferromanganese production (after controls)			
Blast furnace cast house[d]	230–820	1,100–22,600	Ruhf (1978)
Pig casting	390–620	3,960–5,200	Ruhf (1978)
Mn processing	390–2,260	1,000–24,300	Ruhf (1978)

[a]All studies prior to 1978 were associated with evidences of manganese toxicity in some individuals.
[b]Methods of sample collection differ, i.e., thermal precipitation, electrostatic precipitation, millipore filter sampling.
[c]Values later found to be too low.
[d]F = fume.

RELEVANT ASPECTS OF MANGANESE METABOLISM

General considerations. Manganese shares with many other metals the properties of being essential to health and also being toxic in high concentrations. Fundamental to any consideration of possible harmful effects from environmental exposures to manganese is whether or not the manganese intakes are outside an optimal range between deficiency and toxicity. The body has very efficient homeostatic mechanisms, mediated largely through controlled excretion by way of the liver, bile, and intestinal tract, which can maintain Mn balance despite wide variations in daily intake. The latter is largely from food, as will be pointed out later. There is evidence that variable absorption may also be a factor in homeostasis (Abrams et al., 1976).

Evidence for essentiality. The evidence for manganese being essential to life has been conclusively demonstrated in several species of lower animals

and plants (National Academy of Sciences, 1973; Mertz, 1981). The specific functions for humans are, however, still poorly defined. One case has been reported by Doisy (1973) in which deficiency was suspected of causing clinical symptoms. He described an instance where a volunteer in an experiment was given a diet in which Mn was inadvertantly omitted. The volunteer developed mild dermatitis, reddening of his hair and beard, nausea, lowered serum cholesterol, and depressed synthesis of glycoproteins, all of which cleared when Mn was provided.

Some of the most striking effects seen in animals on Mn-deficient diets have related to reproduction (Everson et al., 1959; Apgar, 1968; National Academy of Sciences, 1973). Hurley (1981) recently has summarized the evidence that Mn, as well as copper and zinc, is essential for normal prenatal and neonatal development, with deficiencies resulting in a variety of congenital abnormalities.

Manganese intake. The safe and adequate dietary allowance of manganese for an adult recommended by the National Academy of Sciences is 2500 to 5000 μg/d (National Academy of Sciences, 1980; Mertz, 1981). A World Health Organization task force has estimated that average daily intakes by adults range from 2000 to 9000 μg (1981). The most widely accepted figures for the estimated average daily intake is 3000 μg, but Schroeder et al. (1966) stated that a more typical intake in the United States at that time was slightly less. They believed that the refining of grains and sugars had resulted in human intakes being at the margin of manganese deficiency. For example, data were quoted to indicate that a change from white flour to whole wheat flour could increase an adult's dietary intake of Mn from 2200 μg to 8500 μg/d.

Most manganese enters the body from the gastrointestinal tract and is derived from food, with water and air contributing only a small fraction. The World Health Organization task force estimated 0.5 to 200 μg absorbed from water and from less than 2 to 10 μg/d from inhalation. The inhalation intake depends largely on whether a population lives near manganese-emitting industries, especially ferromanganese or silicomanganese operations.

Guthrie and Robinson (1977) reported a study of diets in 23 women in New Zealand and concluded that a daily intake of 2700 μg was typical for nonvegetarian Western diets. On the other hand, Wenlock et al. (1979) found that the average British diet provided 4600 μg Mn/d, of which half came from tea and other beverages.

The absorption of ingested manganese in adults has been estimated at from 3 to 10%, with the lower percentage being widely accepted as being applicable to healthy adults (Matrone et al., 1977).

Studies of Mn intake in human neonates and infants have largely been concerned with the possibility of manganese deficiency (Shaw, 1980). Vuori (1978) has reported on Mn in human milk where the average ranged from 4 to 6 μg/l, with a decline from initial values of 5.9 μg/l to about

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4 μg/l before the second month and rising again about the fifth or sixth month after birth of the child.

Reference Man, a hypothetical 70-kg adult of middle age, contains about 12,000 μg manganese in his entire body, the range being 10,000 to 20,000 μg (World Health Organization, 1981). The concentrations in human tissues do not increase with age (Schroeder et al., 1966). This reflects the excellent homeostatic mechanisms, which keep tissue levels relatively constant despite considerable variations in intake.

Comparison of pulmonary and gastrointestinal absorption. Mouri (1973) studied manganese absorption in mice that inhaled manganese dioxide dust, in which 96.8% of the particles were less than 3 μm in diameter, in concentrations averaging 8910 μg Mn/m^3 and 5550 μg Mn/m^3 for 2 h/d for 8 d and 15 d, respectively. In spite of the high concentrations, no changes were observed in the appearance, body weight, or food intake of the animals.

At both levels of inhalation exposure, the concentrations in most tissues or organs were reported as being higher following inhalation than in controls on comparable oral intakes of manganese or in animals that had ingested much higher amounts of manganese. However, the major differences were in the lung and trachea and in the stomach and small intestine, differences that appear consistent with local deposition and the swallowing of inhaled particles. The relatively small differences in the ratios (inhalation/ingestion) of accumulated manganese in the kidney (1.6 and 1.3), pancreas (1.1 and 1.4), spleen (0.6 and 0.8), brain (1.3), bone (0.95), muscle (2.0), and blood (0.9 and 1.1) provide excellent evidence for the ability of the mice to regulate manganese when inhaled, in spite of the massive doses to which they were exposed.

Adult Swiss mice exposed by *inhalation* to 70,000 μg of MnO_2 dust/m^3 for 35 h/wk for 4-9 mo (Morganti et al., 1982) showed altered behavioral patterns when compared with controls and with animals that had *ingested* comparable amounts of Mn. The effects were relatively minor, however, and were accompanied by more rapid growth and increased numbers of offspring in some exposed groups. The authors suggested that the latter effects might have reflected a suboptimal Mn content in the laboratory diet.

Retention and distribution of Mn in younger animals. There is experimental evidence that very young rats show higher absorption of ingested manganese than adults and that the retention in the brain is greater. Mena (1974) says this is of the order of 70%, compared with less than 3% in adults, and Rehnberg et al. (1980, 1981, 1982) concurred in these estimates.

Kostial et al. (1978) have provided convincing experimental evidence to demonstrate the increased absorption of radioactive manganese chloride in young rats. Gastrointestinal absorption was 39.9% in 1-wk-old animals, as compared with 0.4% in 6-wk-old rats on a milk diet and 0.05% in 6-wk-old rats on a standard diet (which contained considerably more manganese).

There was a significant difference in tissue distribution in sucklings and older rats. However, these changes were not reflected in differences in acute toxicity. The LD50 for $MnCl_2$ was essentially the same at 2 wk and at 18 wk, the values in mg/kg being as follows: 2 wk, 804; 3 wk, 1860; 6 wk, 1712; 18 wk, 850; and 54 wk, 619. The authors concluded that while the data did not support the hypothesis of increased sensitivity to metals in the newborn, it was still highly undesirable to create a high body burden in infancy.

The most pertinent recent work on the retention and distribution of ingested manganese in very young animals is that of Cahill et al. (1980). Their studies used $^{54}Mn_3O_4$ and $^{54}MnCl_2$ and showed that the two compounds displayed different retention and distribution patterns. With the chloride, retention at a dosage of 500 μg Mn/rat was 13.4%, contrasted with 1.1% for the oxide. At 25 μg Mn/rat, the percentages retained were approximately the same, 20.1% and 17.9%. The most striking findings reported by Cahill et al. were that at a single oral dosage of 25 μg, retention peaked at 10 d, with 22% retention, and then declined precipitously through d 19. At 24 h after exposure, infant rats had from 2 to 65 times greater brain concentrations than did adolescent or adult rats, and these remained higher even after 25 or 49 d. At low doses, nearly 50% of ingested and retained Mn was in the liver, but at higher doses—e.g., 500 μg/d, the proportion was much less, suggesting to the authors that higher doses overloaded the liver's sequestering power. These studies provide valuable information that leads to the conclusion that a doubling of the average daily intake of Mn in an infant could not be tolerated as well as it would be in an adult after the necessary Mn reserves had been established. However, the increments in Mn intake that would result from MMT use would be only a minute fraction of an average daily intake from food and background Mn in water and air.

Kontur and Fechter (1982) administered $MnCl_2$ to rats in doses of 0, 25, and 50 μg/g via intubation from birth through 21 d. Mn levels in the brain increased twofold to threefold with evidence that excretion began between 14 and 21 d. There were no effects on growth nor any overt behavioral or neurochemical toxicity. They concluded that the neonatal animal is not particularly susceptible to the neurotoxic effects of Mn.

Susceptibility of older animals. There are studies to suggest that very old animals also may be unusually sensitive. Thus, Silbergeld (1982) has shown that rats 24-32 mo old showed significantly lower straital dopamine levels than did rats 2-3 mo old, when both groups were given manganese acetate at 5 mg/l in drinking water. As with most other experimental findings of importance in understanding manganese toxicity, levels of exposure were greatly in excess of those relevant to MMT use.

Retention and absorption in relation to iron intake. Mena et al. (1974) suggested that individuals with iron-deficiency anemia might be vulnerable because of increased intestinal absorption. The evidence to support this is

limited to feeding studies with large doses of Mn. The order of increase as shown by Mean et al. (1969) was 3% absorption in normal individuals, compared with 7.5% absorption in anemic subjects. Chandra and Tandon (1973) found that rats on iron-deficient diets given 10,000 μg manganese chloride/kg daily for 15 d had higher manganese content in liver, kidney, and testes than those on a normal iron diet. The ratios were 1.3 to 1, 1.4 to 1, and 1.6 to 1, respectively. Rehnberg et al. (1982) have confirmed the foregoing in studies in which rats ingested Mn_3O_4 in diets containing 400, 1100, or 3550 μg Mn/g. Iron-deficient diets promoted Mn absorption and tissue accumulation. These findings, although important, do not seem relevant to the addition of a few micrograms of Mn to the several thousand micrograms present in the average diet.

Absorption and retention of Mn from MMT combustion. There are several studies in animals that involve the absorption and tissue localization of manganese derived from MMT. Moore et al. (1975) reported the exposure of rats and hamsters 8 h/d for 56 d to automotive emissions containing Mn particulates that had resulted from the use of MMT as an additive. Both irradiated and nonirradiated exposure chambers were used. The amounts of manganese in the air of the chambers were 117 and 131 $\mu g/m^3$, respectively. These concentrations were more than 200 times the predicted increments in airborne Mn predicted along urban street corridors from the use of MMT as a gasoline additive, and over 4000 times the predicted median increases in ambient air concentrations. Tissue concentrations of Mn in the brain and liver were increased in test animals at the end of 24 wk; the concentrations in the brain were $1\frac{1}{2}$ to 2 times those found in control animals. There were no histopathologic changes.

Ulrich et al. (1979a,b,c) described studies in which rats and squirrel monkeys were exposed to inhaled manganese oxide aerosol produced by the combustion of MMT, with average exposure concentrations of 11.6, 112.5, and 1152 $\mu g/m^3$ 21-22h/d, for 9 mo. While concentrations of manganese in liver, kidney, pancreas, spleen, lung, and blood were elevated after 9 mo, when measured 6 mo after exposure ended they were not elevated in any groups. Brain Mn concentrations were not determined.

Coulston and Griffin (1976) exposed rhesus monkeys to a manganese oxide aerosol, also derived from the combustion of MMT, where concentrations were approximately 100 μg Mn/m^3 for periods up to 66 wk. They calculated that the diet of the rhesus monkey contained between 4000 and 5000 μg Mn/d and that there was about 100 μg Mn in all the air inhaled by an animal during each day in the chamber. No toxic effects were observed. In animals sacrificed after 12 mo of exposure, there were slight but statistically significant increases in the manganese concentrations in lungs, liver, pancreas, kidney, and heart muscle. Concentrations were also greater in the pallium cortex, basal ganglia, cerebellum, and pons; the degree of difference was about two-fold with the exception of the pons, which had about four times the amount of manganese in the exposed

animals than in the control animals. This study confirmed that prolonged exposure by inhalation for over a year to concentrations of manganese far greater than the peaks predicted for MMT use led to increases in manganese in the brain, but no symptoms appeared.

The same authors also reported that in rats exposed to airborne manganese from MMT combustion, in concentrations of 100 $\mu g/m^3$ for up to 8 wk, there were increased amounts of Mn in the lung and brain, but that these returned to normal levels within 1 wk after cessation of exposure.

In summary, it appears that the most relevant studies bearing on the possibility of increased accumulation of Mn in tissues resulting from the use of MMT are those that have been done with the actual combustion products. Here, even when exposures involved concentrations exceeding by several orders of magnitude any that would occur in heavily travelled urban areas, increased Mn levels in tissues were relatively low and rapidly returned to pretest levels.

POTENTIAL FOR CENTRAL NERVOUS SYSTEM EFFECTS

Human experience. A disease of the central nervous system resembling Parkinsonism is a distinctive manifestation of chronic manganese poisoning. It is a well-recognized occupational disease that has been the subject of many clinical reports and studies (Flinn et al., 1940; Rodier, 1955; Penalver, 1955; Khazan et al., 1956; Schuler et al., 1957; Whitlock et al., 1966; Emara et al., 1971; Smyth et al., 1973; Hine and Pasi, 1975). In none is there convincing evidence that such chronic manganism occurred in anyone whose inhalation exposures had not exceeded 5000 $\mu g/m^3$ over fairly long periods of time.

There are a few questionable reports of chronic manganism with lower exposures. Whitlock et al. (1966) attributed two cases of chronic neurologic disease to exposures of less than 5000 $\mu g/m^3$. However, Tanaka and Leiben (1969) subsequently reported that the average concentrations of Mn in some areas of the plant in question were 11,000 $\mu g/m^3$ with peaks over 30,000 $\mu g/m^3$. Smyth et al. (1973) described 5 cases of chronic manganism in 71 workers in a Pennsylvania steel plant. One had worked in an area with exposures to ferromanganese fumes averaging only 1000 μg Mn/m^3, which led the authors to hypothesize and unusual hypersusceptibility. A recent report by Chandra et al. (1981) of suspected neurological involvement in welders exposed to 440-2600 μg manganese/m^3 has insufficient clinical information to justify firm conclusions. One can conclude that in the range of exposure from 1000 to 5000 μg Mn/m^3, chronic manganism may occur but has not been clearly proven.

Even with high concentrations in the air, not all workers show neuro-

logic symptoms or signs. For example, Lloyd-Davies and Harding (1949) studied workers in a plant where exposures ranged as high as 21,600 $\mu g/m^3$ and found that "no case of systemic manganese poisoning was seen in spite of the closest watch" (p. 89).

The American Conference of Governmental Industrial Hygienists (ACGIH) (1980) in its documentation for the threshold limit value (TLV) for manganese dust and manganese compounds concluded that no evidence of adverse effects had been documented for workers exposed to manganese dust in concentrations averaging below 5000 μg Mn/m^3, a level that it felt afforded a margin of safety. It regarded manganese tetroxide and fume as more hazardous and recommended for them a more restrictive TLV, i.e., 1000 μg Mn/m^3 as a TWA, with 3000 μg Mn/m^3 as a short-term exposure level (STEL) for the fume.

There is little information on tissue levels in humans associated with neurologic disease. Analyses that have been reported have not shown any correlations. For example, Flinn et al. (1940) reported an autopsy on an individual with manganism, 7 yr after exposure, which had averaged 73,000 μg Mn/m^3 air. There were excess amounts of Mn in the lungs but not in other tissues. It has been suggested by Cotzias (1958) that it is the perfusion of the brain during high turnover of Mn, rather than its storage, that is important. This might occur in individuals who have stored large amounts of Mn in their lungs during industrial dust exposures.

In a systematic study of trace metals in human tissue samples, Schroeder et al. (1966) reported excessive Mn concentrations, more than five times the mean for the organ, in the brains of four individuals who did not have increased concentrations in other organs. Several others showed high concentrations with concurrent elevations in other organs. There were no cases of Parkinsonism in this series.

Studies of central nervous system effects in lower animals. No satisfactory small-animal models have been developed for studying symptomatic extrapyramidal disease caused by manganese. Mice, rats, and guinea pigs have proved useful, however, for studies of metal deposition and neurochemical effects (Bull, 1977; Chandra et al., 1979a,b, 1980; Deskin et al., 1980a,b; Hietanen et al., 1981; Shukla and Chandra, 1977, 1979, 1981, 1981; Papavasilou et al., 1975; Donaldson et al., 1980; Donaldson and La Bella, 1981; Seth and Chandra, 1981). A number of these studies used weanling rats and provided evidence that neurochemical changes were produced with smaller doses than required for adult animals (Chandra and Shukla, 1978; Deskin et al., 1980a; Kostial et al., 1978; Seth et al., 1977). Although the amounts of manganese administered were relatively large—e.g., 5-20 mg/kg·d—toxic symptoms and signs were not apparent in most studies. Mice, rats, and guinea pigs are not subject to typical extrapyramidal tract disease, presumably because they do not have pigmentation in the substantia nigra. Mild behavioral changes in mice were described

in one study (Chandra et al., 1979a) when pups whose nursing mothers had been given a 5-mg/ml $MnCl_2$ solution (about 30 ml/d) and were later given increased Mn in drinking water after weaning, showed enhanced motor behavior at 60 and 90 d.

Although these studies provide interesting models for studying the influences of Mn on biogenic amines and a variety of neurologically important enzymes, they are only marginally relevant to the toxicity of MMT combustion products because the levels of Mn exposure were greater by orders of magnitude than any increments from the use of MMT.

Primates develop extrapyramidal disease manifestations from Mn and can be used experimentally, as first shown by Mella (1924). As an example, Neff et al. (1969) have shown that squirrel monkeys that received MnO_2 by injection in total dosage of 2000 μg developed neurological signs and reductions in caudate dopamine.

Dastur et al. (1969) studied intraperitoneally administered ^{54}Mn in rats and concluded that Mn was taken up very slowly by the central nervous system as compared with other organs, but that it was also retained longer. The periods of observation were 34 d. The authors suggest that this avidity of CNS tissue for manganese might be the cause for the vulnerability of the CNS in chronic manganism. This is a plausible hypothesis when dealing with heavily exposed workers, but it is probably not relevant to the addition of a few micrograms of manganese per day in individuals already handling several thousand micrograms. Even though the half-life of Mn is longer in CNS tissue than in most other tissues, there is no buildup with age at levels of intake in the range of the average diet.

Toxicologic studies of MMT combustion products. The studies in animals most relevant to the use of MMT have been those of Ulrich et al. (1979a,b,c) and those of Coulston and Griffin (1976), which were mentioned in the section on absorption and tissue accumulation. The former workers reported studies in which rats and squirrel monkeys inhaled manganese oxide aerosol produced by the combustion of Mn, with average exposure concentrations of 11.6, 112.5, and 1152 $\mu g/m^3$ almost continuously for 9 mo, with no apparent adverse effects. Specific tests for neurologic damage were carried out in the monkeys.

Coulston and Griffin (1976) exposed rhesus monkeys to a manganese oxide aerosol, also derived from the combustion of MMT, where concentrations of approximately 100 μg Mn/m^3 were maintained for periods up to 66 wk. No adverse effects were observed. The same investigators also exposed two rhesus monkeys to manganese at 5000 $\mu g/m^3$ in an aerosol produced by the combustion of MMT, for 23 h/d for 23 wk. The animals were then observed for an additional 10 mo; there was no evidence of neurotoxicity. The form of manganese inhaled by animals in the latter experiments was largely Mn_3O_4.

Summary of central nervous system effects. There appears to be an extremely wide margin of safety between the airborne concentrations of

manganese that have been associated with central nervous system disease in humans and experimental animals and those that might be found in ambient air resulting from the use of MMT. Experimental animals of susceptible species, when exposed for long periods of time to manganese-containing aerosols produced by the combustion of MMT, have not shown neurologic changes. The concentrations tested in some experiments were very high compared with average increments predicted if MMT were a widely used fuel additive, i.e., 5000 μg Mn/m^3 as compared with 0.025 to 0.05 μg/m^3.

Although manganese is probably more readily absorbed in infants and crosses the blood-brain barrier more readily, all available evidence would indicate that the amounts of Mn added to ambient air by MMT would fall within the range of normal daily absorption and would not cause neurologic effects, even in the most susceptible portion of the population.

EFFECTS ON THE RESPIRATORY TRACT

Human occupational exposures. Acute effects on the lungs, variously described as pneumonia or pneumonitis, have been reported in industrial workers exposed to manganese (Brezina, 1921; Lloyd-Davies and Harding, 1949; Rodier, 1955; Šarić et al., 1974). In these reports, where manganese concentrations were measured, the peaks have ranged from 16,300 μg/m^3 to 926,000 μg/m^3, so that these occurrences are really irrelevant to the proposed use of MMT. It is of interest that in many other work situations where Mn concentrations were in the same range, no pulmonary effects were described. This suggests that there is some covariable, possibly an infectious agent, that explains the pneumopathies.

Human nonoccupational exposures. Community experience is not clear-cut. The oft-quoted report of Elstad (1939), based on experience between 1924 and 1934 in the Norwegian town of Sauda, provides circumstantial evidence suggesting an association between atmospheric pollution and lobar pneumonia, but it cannot be definitely related to manganese, which was present in concentrations above 64 μg/m^3. Studies by Povoleri (1949, 1969) in Aosta, Italy, are inconclusive and cannot be used as evidence. Studies from Japan, reported by Nogawa et al. (1973), Kagamimori et al. (1973), and Yoshikawa et al. (1973) have been reviewed. While they suggested that airborne manganese in the range of 4-7 μg/m^3 resulted in increased respiratory symptoms, pneumonia, or impaired pulmonary function in school children, these reports have serious deficiencies. There is uncertainty as to the actual levels of airborne Mn, the matching of groups socioeconomically, how biasses in questionnaire replies were eliminated (in view of the respondents' concern about the possible harmfulness of air pollution), and how the role of atmospheric factors other than manganese was evaluated. The Environmental Protection Agency concluded that in these studies, the critical level of Mn was "not well identified in a quantitative

sense" (U.S. Environmental Protection Agency, 1975). The brief reports by Suzuki (1970), based on questionnaire surveys, share some of the same deficiencies and are too limited for evaluation.

Šarić et al. (1975) studied acute respiratory disease in the town of Sibenik on the Dalmation coast, comparing the incidence of acute bronchitis, peribronchitis, and pneumonia over a period of 3 yr in three zones at varying distances from a manganese alloy plant. Mean yearly concentrations of manganese in zone I, nearest the plant, ranged from 0.271 to 0.438 $\mu g/m^3$; in zone II, from 0.176 to 0.254 $\mu g/m^3$; and in zone III, 0.051 to 0.070 $\mu g/m^3$. Weekly averages varied widely, with the maximum values for zones I, II, and III being 1.241, 1.323, and 0.251 $\mu g/m^3$, respectively. There was a slightly greater incidence of acute bronchitis and peribronchitis in the two zones nearer the plant, but no difference in the annual incidence of pneumonia. There was not the expected difference between summer and winter rates for pneumonia, and the authors speculated that this might relate to higher Mn concentrations in summer. The authors regarded their findings as tentative, in view of the impossibility of controlling for population density and the absence of information on air pollutants other than manganese and sulfur dioxide.

Studies in lower animals. Bergstrom (1977) reviewed the literature on the acute pulmonary toxicity of manganese and described experimental studies in guinea pigs exposed to MnO_2 at concentrations of 22,000 $\mu g/m^3$ where 87% of the particles were greater than 3 μm in diameter. He found primary inflammation of the bronchi of limited duration, and a significant decrease in bacterial clearance.

There are limited data supporting alteration of resistance to bacterial and viral pneumonia by exposure of mice to MnO_2 aerosols in concentrations of 109 $\mu g/m^3$ (Maigetter et al., 1976).

Adkins et al. (1980b) found no significant pulmonary edema in mice exposed by inhalation to Mn_3O_4 for 2 h in concentrations of 1837 μg Mn/m^3 and no mortality in mice exposed for 2 h to Mn_3O_4 aerosols ranging from 1583 to 2599 μg Mn/m^3. However, pulmonary cells in mice exposed to Mn_3O_4 in concentrations of 897 μg Mn/m^3 for 2 h showed biochemical and enzymatic changes consistent with slight impairment of defense mechanisms (1980a).

In none of the major studies involving exposure of animals to manganese oxides derived from MMT—e.g., those of Moore et al. (1975), where concentrations were 117 $\mu g/m^3$ for 56 d; Coulston and Griffin (1976), where concentrations were 100 $\mu g/m^3$ for many months; and Ulrich et al. (1979a, b,c), with concentrations exceeding 1000 $\mu g/m^3$—were any respiratory-tract problems described.

From review of all of the evidence, it appears extremely unlikely that minute increments in airborne manganese even as great as 1 or 2 $\mu g/m^3$ would have any detectable effect on the lungs.

OTHER POSSIBLE EFFECTS

The cardiovascular, hematopoietic, and reproductive systems have been mentioned in the literature as the sites of possible toxic effects of manganese. Manganese has also been studied for its mutagenic and carcinogenic potential.

Some manganese salts induce a fall in blood pressure in experimental animals (Kobert, 1833; Schroeder and Perry, 1955; Schroeder et al., 1955), ar.. there is one study reporting that groups of heavily exposed workers isplay slightly lower average systolic pressures (Šarić and Hrustic, 1975).

Hematopoiesis. Iron and manganese compete for intestinal absorption, and rats fed high concentrations of Mn in their diets and low iron intakes show more evidence of iron deprivation than animals with normal manganese diets (Carter et al., 1980).

Reproduction. There are a variety of adverse effects on reproduction associated with manganese deficiency (Everson et al., 1959; Apgar, 1968; National Academy of Sciences, 1973). Very high amounts of manganese, on the other hand, can produce testicular changes, retarded sexual development, and other reproductive effects in rabbits, mice, and rats (Imam and Chandra, 1975; Gray and Laskey, 1980; Laskey et al., 1982).

Mutagenicity. High concentrations of Mn are mutagenic in some *in vitro* test systems (Durham and Wyss, 1957; Orgel and Orgel, 1965; Miyaki et al., 1977; Kaplan, 1962; Putrament et al., 1975; Dube and Loeb, 1975), but not in others (Simmon and Ligon, 1977). These effects may reflect interference or competition with other essential metals.

Carcinogenesis. The possible role of manganese as a carcinogen has been reviewed by Kazantzis (1981). DiPaolo (1964) reported that 67% of DBA mice developed lymphosarcomas after 18 mo of manganese chloride administration, compared with 24% in controls. Stoner et al. (1976) reported a slight increase in lung tumors in mice given manganous sulfate at 660 mg/kg intraperitoneally over a period of 30 wk. Fürst (1978) found no excess tumors in rats given manganese powder (10 mg × 24 oral doses). There wre also no effects in mice.

Manganese maleate has been reported as inhibiting new growths with both Ehrlich's tumor in the mouse and Gueri's tumor in the rat [Balo and Banga (1957), reported by Cotzias (1958)]. Sunderman (1977) and Sunderman et al. (1975) showed manganese to be an inhibitor of the development of fibrosarcomas produced by nickel subsulfide.

There are no reports to suggest that manganese is a human carcinogen; in fact there are two studies (Majanen and Soini, 1972; Schrauzer et al., 1977) suggesting an inverse relationship between manganese intake and the incidence of some types of cancer.

In summary, with respect to effects on the cardiovascular, hematopoietic, and reproductive systems, there is no evidence to suggest that small increments in environmental manganese from the combustion of

MMT would have any impact on health. The same is true with respect to mutagenic and carcinogenic effects.

RECAPITULATION

The questions raised in the introduction to this report have been considered in the light of available environmental, experimental, and epidemiologic data, with the following conclusions:

1. The increments in manganese intake in humans resulting from the use of MMT would be within the physiologic range and far below those shown to be toxic.
2. While there are differences in the absorption, distribution, and excretion of manganese that is inhaled as contrasted with manganese that is ingested, the differences are relatively small. Animals that have inhaled manganese derived from the combustion of MMT in concentrations greatly in excess of any that would result from MMT's use as a gasoline have not shown toxic effects.
3. There is moderately increased absorption of Mn associated with iron-deficiency anemias, but this relates to ingested Mn. Any increments in ingested Mn from MMT use would be within the variations that normally occur from differing dietary intakes.
4. Very young experimental animals have increased intestinal absorption of Mn and poorly developed blood-brain barriers to metals. While this suggests that they might be hypersusceptible to central nervous system effects from manganese, the increments of Mn from MMT use would lie within a range to which they are already being exposed, and far below concentrations where such hypersusceptibility would be operative.
5. There is also no evidence to support any discernible impact of minute increments of Mn from MMT on the respiratory tract, the cardiovascular system, hematopoiesis, or reproduction. Neither should mutagenic or carcinogenic effects be anticipated, in view of the fact that tota! Mn intakes would remain in the physiologic range essential to health.
6. In spite of the fact that there are gaps in our knowledge of the metabolism of manganese and its functions and effects in biologic systems, these are more than balanced by experimental studies with high concentrations of Mn derived from the combustion of MMT. The minute increments of Mn that would result from the use of MMT as a gasoline additive should not have any impact on the public's health.

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ATTACHMENT D-1

A Review of the Memorandum Entitled, *"Manganese in Gasoline, Toxicity Problems"* by the Director, NIEHS dated June 7, 1990

Carl O. Schulz, Ph.D., DABT

In his memorandum, dated June 7, 1990, the Director of NIEHS expressed concern regarding the proposed use of manganese-containing compounds as additives in gasoline. The memorandum presents reasons for that concern. The questions raised show misunderstanding of the chemical and biological properties and behavior of manganese and its compounds and do not reflect the large body of scientific information on manganese.

In paragraph 1, the concern is raised that manganese is toxicologically similar to lead. This is not scientifically supportable and is addressed in more detail in a separate document. The Director also indicates concerns regarding organic and inorganic forms of manganese. Since the available data indicate that there are no organic compounds of manganese present in the exhaust from engines fueled with gasoline containing MMT (manganese is emitted as manganese oxides with Mn_3O_4 predominating [Ter Haar et al. 1975] - JAPCA 25:858-860), this proceeding addresses only the potential hazards to human health of inorganic forms of manganese.

In paragraph 2 the Director speculates, without evidence, that MMT is readily absorbed "via the nose" and that this might result in higher levels of manganese in the CNS than comparable doses by other routes. As stated above, the manganese is emitted in engine exhaust as inorganic oxides, mostly presumably in particulate form. Depending on particle size, a significant but unknown proportion of the manganese that enters the respiratory tract will deposit on the surface of the upper respiratory tract and will be expelled or cleared to the gastrointestinal tract by normal mucociliary clearance mechanisms. There is no evidence, and it is unlikely, that a significant amount of manganese will be absorbed through the mucosa of the nasopharyngheal region of

the upper airway. In any event, this does not guarantee ready access to the brain. Absorption would occur into the general circulation and the blood supplying the capillary bed of the nasopharyngheal mucosa must pass back through the heart, lungs, and probably the kidneys and liver before it reaches the brain. There is no shortcut from the nose to the brain as implied in this paragraph.

The Director points out that most of the toxicity data are by the oral route of administration and do not apply to other routes. This generalization applies only to experimental studies. There is a large body of health effects information derived from studies of workers who are occupationally exposed to manganese compounds almost exclusively by the inhalation route (see HEI 1988, Cooper 1984, EPA 1984, and WHO 1981 for reviews of these studies).

In paragraph 3 the Director asserts that the effects of manganese on the central nervous system are not reversible "easily, if at all". However, the World Health Organization concluded that the neurological damage attributable to manganese exposure is reversible if the patient is removed from exposure at an early stage and that the symptoms of manganism can be treated by administration of L-Dopa (WHO 1981).

In paragraph 5 the Director states that Mn_3O_4 is much more toxic than MnO. While no documentation is provided for this assertion, it is probably based on the acute oral toxicities of these compounds in laboratory animals. The relevance of acute toxicity to the relative human health hazards of these two forms of manganese resulting from chronic exposure to low concentrations in ambient air is doubtful. Moreover, because, as stated above, the toxicity of MMT is not at issue here, the relative toxicity of MMT and tetraethyl lead is irrelevant.

The point of paragraph 6 is not apparent. Epidemiologic studies are incapable of detecting low incidences of subtle adverse health effects because of the lack of statistical power. Ordinarily such studies are conducted in relatively small cohorts of highly exposed individuals in order to maximize the probability of a statistically significant outcome. It is asking

too much of epidemiologic methods to rely on such studies to provide quantitative dose-response data within the range of expected environmental exposures.

The argument raised in paragraph 7 is purely speculative and once again raises the issue of toxicological parallels between manganese and lead. These issues are dealt with in a separate memorandum.

In conclusion, the memorandum from the Director of NIEHS fails to provide a scientific basis for any of the concerns raised therein regarding inorganic forms of manganese, and does not provide a basis for rejecting the data on which Ethyl Corporation bases its request for approval of a manganese additive in gasoline.

ATTACHMENT D-2

Contrasting Public Health Concerns Raised by Lead, Manganese, and MMT

By Carl O. Schulz, Ph.D., DABT

In the memorandum from the Director of NIEHS it is implied that manganese and lead are similar elements presenting similar public health problems. Moreover, this memorandum and the letter from Dr. Needleman imply that organic compounds of Mn and Pb have similar toxicities. The only characteristics shared by lead and manganese are that they are metals, form compounds in which they exist in the +2 and +4 oxidation states, and may adversely affect the central nervous system under certain conditions. The differences between them chemically, biologically, and environmentally are many and profound.

Chemically, lead is a "heavy" metal, atomic weight 207, in group IVA of the periodic table. Manganese on the other hand is a "light" metal, atomic weight 55, in transition group IIIA. The chemical behavior of manganese resembles that of its fellow transition metals chromium and iron much more than lead. (Pauling 1964). There are 11 valence states of manganese, 8 of which are oxidation states. The most stable of these (+2 and +3) occur in the environment; only the +2 oxidation state of lead is common in naturally occurring compounds. (Grayson, 1985, pp. 728-730).

Environmentally, manganese is the twelfth most abundant element in the earth's crust and is present in soil, water, and in all plant and animal tissues. (Cooper, 1984). Humans are exposed to relatively high concentrations of manganese in food, water and air even in the absence of pollution. (WHO, 1981). Lead, on the other hand, is present in the earth's crust at concentrations 1 to 2 orders of magnitude below those of manganese (IARC, 1980), and in the absence of anthropogenic sources of lead pollution is present only at low levels in the human environment.

Biologically, the differences between lead and manganese are even more striking. Manganese is an essential trace element in human and animal nutrition. (NAS, 1973; WHO, 1981). Lead has no known beneficial role in biological systems. (Goyer, 1986). Homeostatic mechanisms appear to regulate the uptake and excretion of manganese in higher animals such that individuals having widely different intakes of this element have similar body burdens. (HEI, 1988; Cooper, 1984). There are no known mechanisms that regulate the uptake of lead. Manganese is readily excreted from the body in a biphasic manner. (WHO, 1981). The "slow" phase has a half life on the order of 38 days. Although there is some evidence that manganese levels in the brain decrease at a slower rate than those in the rest of the body, there is no evidence that manganese accumulates in the brain at "normal" environmental exposures. While some lead is eliminated from the body rapidly, much of the lead to which humans are exposed is sequestered in bone, where it has a half-life of more than 20 years, and other tissues, from which it is eliminated very slowly, if at all. (Goyer, 1986). Lead affects the peripheral and central nervous systems. While the mechanism is not clearly understood, it is clear that the function of the nervous system in its entirety is involved. Manganese, on the other hand, appears to affect the extrapyramidal motor system in the basal ganglia in the central nervous system, possibly through interference with dopamine metabolism. (HEI, 1988; Bleecker, 1988). The concentrations required for this to occur appear to be far above "normal" concentrations and the effects may be modulated or eliminated by the administration of dopamine precursors. Limited evidence indicates that the central nervous system effects of manganese are reversible, at least in the early stages of intoxication. The absence of neurological impairment associated with normal environmental exposures to manganese, the limited evidence for reversibility, and the effective treatment of early symptoms with therapeutic agents support the possible existence of a threshold for the neurological toxicity of manganese. No threshold has as yet been established for the toxic effects of lead on the central nervous system.

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ATTACHMENT D-3

A Brief Comment on *"Manganese and Human Health"* by John Donaldson in Manganese in the Canadian Environment by the National Research Council Canada, 1988

Carl O. Schulz, Ph.D.

This document is neither a research article nor an objective scientific review article. It is an extended essay in which the author lets his imagination run free, citing mere wisps of scientific evidence in support of a grandiose hypothetical scheme of manganese toxicity. It is fair to say that the only type of peer-reviewed scientific journal that would publish such a speculative piece would be a journal devoted to scientific hypotheses. As support for the hypothetical nature of the document one has only to analyze the language as follows:

Page	Phrases (emphasis added)
90	"and may be therapeutically useful..."
	"consideration should be given to the..."
	"Possible sites may be free carboxyl..."
91	"may have considerable relevance towards..."
	"seems to indicate that environmental..."
	"may play a previously unsuspected..."
	"manganese may contribute..."
	"which may not completely manifest itself..."
92	"it is considered by some investigators that..."
	"and possibly some other neurodegenerative..."
	"manganese may induce initial sub-clinical..."

93 "Calne et al. 1986) have suggested that..."
"they have extended this hypothesis to..."
"The practical implications of this intriguing hypothesis strongly suggest that..."
"A prime candidate for examination in causal mechanisms linking..."

94 "Certainly the possibility of initial..."
"it should be possible to determine..."

This type of analysis throughout the document reveals the purely speculative nature of the author's arguments. However, the author writes his own bottom line on page 92.

> "Whether manganese can in fact induce an [sic] lesion in discrete and vulnerable compartments of the CNS during the early years and which does not result in neurotoxicity until later stages of development is presently unknown. However, because manganese is an important neurotoxin whose precise mode of nervous tissue and behavioral toxicity is still unknown, it should be considered a prime candidate in studies set up to examine this specific question. Also, the concept that manganese may induce initial sub-clinical neuronal damage which remains dormant for decades until late life when as a result of potentiated senescence changes, it results in a chronic neurologic disorder such as Parkinson's disease, Alzheimer's disease, or amyotrophic lateral sclerosis (ALS) has not yet been adequately addressed." (Emphasis added)

One wonders why the author did not also include a number of other neurological diseases of unknown etiology, e.g. multiple sclerosis and muscular dystrophy, on his list of disorders attributable to manganese. The evidence that he cites in support of an etiologic link between manganese exposure and various neurologic disorders is of the weakest type consisting almost entirely of case reports and ecological studies. It is of

interest also, that the author does not address the fact that while the symptoms of chronic manganese intoxication mimic those of Parkinson's disease, the brain lesion characteristic of manganism can be distinguished from that of Parkinson's disease (Mena et al. 1967; Cooper 1984; and USEPA 1984) [as cited in the HEI 1988 report].

ATTACHMENT D-4

Review of Testimony by Ellen Silbergeld

By Carl O. Schulz, Ph.D., DABT

Dr. Silbergeld testified on behalf of the Environmental Defense Fund (EDF) in opposition to the use of MMT as a gasoline additive. The essence of her opposition was that adding manganese to gasoline is analogous to adding lead to gasoline. This argument is flawed on chemical, biological, and environmental grounds as stated earlier. Dr. Silbergeld cited a study by Davis et al. (1988) as indicating that the use of manganese in gasoline causes an increase in airborne manganese. She failed to indicate that only in a congested urban center did vehicular sources contribute significantly to airborne manganese concentrations and the average contribution of suspended soil exceeded the average vehicular contribution in all locations.

Dr. Silbergeld asserted that "both lead and manganese are elements and as such will not degrade or quickly disappear from stable environmental compartments..." The same can be said about iron, yet no one would assert that iron is like lead. In fact, manganese resembles iron much more closely than lead in that both are transition metals, are abundant in the earth's crust, are essential to human health, and their absorption and excretion are controlled by homeostatic mechanisms. None of these is true for lead.

Dr. Silbergeld asserted, without evidence, that manganese is a cumulative toxin in that both its absorption and retention as well as its toxicity increase with time. Toxicity is an inherent characteristic of a chemical and does not change "with time". With regard to absorption and retention, the EPA concluded in a document for which Dr. Silbergeld was a peer reviewer that "Manganese metabolism is rigorously controlled by homeostatic mechanisms. . . . . The absorption, retention, and excretion of manganese are interrelated and respond very efficiently to an increase in manganese concentration." (USEPA 1984).

Dr. Silbergeld asserted that there are no data on the low-level chronic sequelae of manganese exposure. This statement ignores the fact that humans are continually exposed to relatively high levels of manganese in food and water, and that manganese is an essential element in human nutrition.

Her statement that manganese may accelerate normal cell loss during senescence is a restatement of a speculative hypothesis advanced by John Donaldson and does not recognize the pathological differences between manganese neurotoxicity and other neurological disorders associated with disease and/or aging (see Bleecker 1988).

A-90-16
IV-D-58

BEFORE THE
UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY

IN RE APPLICATION FOR A FUEL ADDITIVE WAIVER FILED BY ETHYL CORPORATION UNDER § 211 (f) (4) OF THE CLEAN AIR ACT	)))))

APPENDICES TO COMMENTS IN SUPPORT OF THE WAIVER APPLICATION FOR THE HiTEC 3000 PERFORMANCE ADDITIVE

VOLUME TWO

APPENDICES 4, 5, 6, 7, 8, 9, 10 AND 11

of Counsel:

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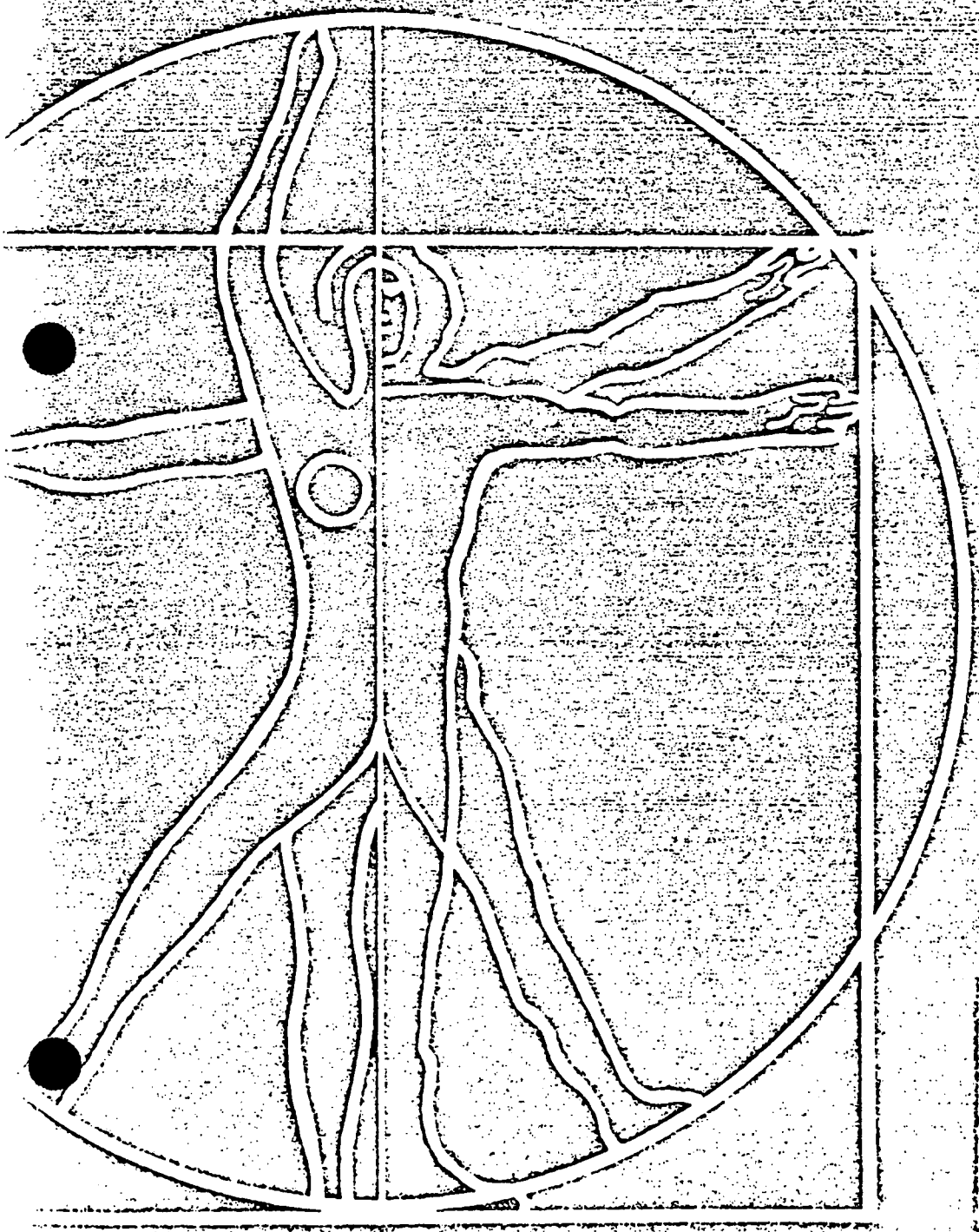
July 23, 1990

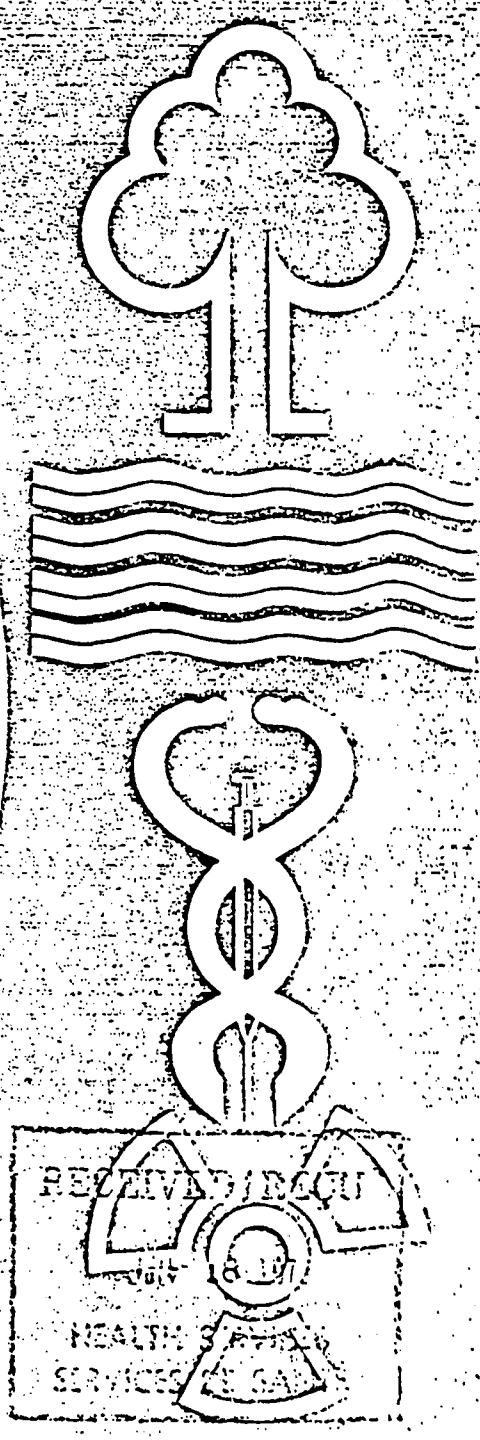
APPENDIX 4

78-EHD-21

Health and Welfare Canada
Santé et Bien-être social Canada

methylcyclopentadienyl manganese tricarbonyl (mmt)





METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT)

An Assessment of the Human Health Implications of its Use as a Gasoline Additive

Environmental Health Directorate
Health Protection Branch

Published by Authority of the Minister of
National Health and Welfare

78-EHD-21

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FOREWORD

This Report was prepared by staff of the Bureau of Chemical Hazards, Environmental Health Directorate, Health Protection Branch, Department of National Health and Welfare. Available information on Methylcyclopentadienyl Manganese Tricarbonyl (MMT) has been reviewed in order to evaluate possible adverse human health effects resulting from its use as a primary antiknock additive in gasoline. This review has been issued as an Environmental Health Directorate publication in anticipation that such a document will prove useful to other government agencies and private groups concerned with ambient air quality.

Acknowledgement is given to Miss M.E. Meek, the principal author, and to Dr. R. Bogoroch for contributing to the preparation of this Report. The provision of extensive information by the Ethyl Corporation is also greatly appreciated.

SUMMARY

At present, Methylcyclopentadienyl Manganese Tricarbonyl (MMT) is added to fuel oil to suppress smoke formation and to improve combustion; current usage of this additive in gasoline is believed to be quite limited. As the amount of unleaded fuel being produced increases, MMT is one additive being given careful consideration as a primary antiknock compound. This Report deals with the possible health implications of its widespread use in gasoline.

MMT is not manufactured in Canada at present. However, its increased use as a fuel additive could result in exposure of individuals involved in the refining and distribution of gasoline. It has been evaluated as safe for intact or abraded skin contact (NIOSH criteria); the American Conference of Governmental Industrial Hygienists Threshold Limit Valve (TLV) is 0.1 ppm - "skin". The "skin" notation is intended to suggest appropriate measures for the prevention of cutaneous absorption, so that the TLV is not invalidated.

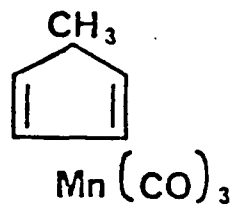
Exposure of the general population to MMT from its use in gasoline would be minimal, since very little (0.1 %) is emitted in the exhaust. The most significant environmental consequence of the use of MMT as a fuel additive is the resulting discharge of manganese to the air, since the principal emission product is Mn_3O_4. Therefore possible health effects of an increase in atmospheric manganese levels are considered. The U.S. E.P.A. estimates that manganese concentrations under worst conditions would increase to less than 5 $\mu g/m^3$ for a 24-hour averaging time. Review of available limited information on industrial and community exposure to manganese and results of studies in animals of chronic inhalation of manganese exhaust products leads to the conclusion that there is no evidence at present to indicate that expected ambient manganese concentrations would constitute a hazard to human health. Data on secondary effects of the use of MMT in gasoline (effects on other emissions and atmospheric reactions) are limited and contradictory; no conclusions can be made at this time about their possible health implications. Recommendations for research to allow a more thorough evaluation of the possible health effects of the use of MMT in gasoline are included.

TABLE OF CONTENTS

I INFORMATION - DATA REVIEW

A. IDENTITY, PROPERTIES, AND ANALYSIS

A.1. Physical and Chemical Properties



Methylcyclopentadienyl Manganese Tricarbonyl
(MMT) (MCMT)
$C_9H_7O_3Mn$ M.W. 218.1

Alternative Names: Manganese Cyclopentyltricarbonyl (MCPT),
Combustion Improver -2 (CI-2),
Antiknock -33X (AK-33X)

Appearance: orange liquid; faint, pleasant, herbaceous odor

Freezing Point (°C)	-2.2
Boiling Point (°C)	233
Melting Point (°C)	1.5
Flash Point (COC), (°C)	93.3°
Specific Gravity (H_2O - 1)	1.38

Vapour Pressure (mm Hg)

20 °C	0.051	mm
25 °C	0.1	mm
61 °C	1	mm
100 °C	9.3	mm
160 °C	100	mm
233 °C	760	mm

Solubility (25 °C)

Water	70 ppm
Glycerine	5 %
N-Hexane	Miscible
N-Heptane	Miscible
Isooctane	Miscible
Toluene	Miscible
Ethanol	Miscible

Thermal Stability: decomposes very slowly at 200 °C and fairly rapidly at 300 °C in inert atmosphere; the presence of oxygen increases the rate of decomposition.

MMT in structurally similar to ferrocene, in that the methylcyclopentadiene ligand is π-bound to manganese. MMT is further classified as a penetration complex because dissimilar ligands are bonded to the manganese atom. The pure compound contains 25 % manganese by weight.

A.2. Analytical Methods

Analysis for MMT in gasoline or liquid fuels generally involves an atomic absorption spectrophotometric determination of manganese.(1)

The U.S. National Academy of Sciences has suggested that procedures now being used for the determination of MMT in gasoline or liquid fuels might be modified to be suitable for its analysis in air.(2) At concentrations above 0.1 mg/l, MMT in air has been determined in one study, by passage through fritted disc absorption towers containing glacial acetic acid and subsequent analysis for manganese by the periodate method.(3) At lower concentrations, it has been determined by passage through absorption towers containing ethyl alcohol and analysis for manganese by the formaldoxime method.(3)

B. USE AND PRODUCTION

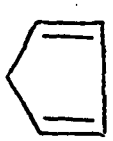
B.1. Use

Methylcyclopentadienyl manganese tricarbonyl is added to fuel oil to suppress smoke formation and to improve combustion. Typical treatment levels are 0.025 g of manganese per gallon of fuel oil for boilers and 0.08 - 0.5 g per gallon for turbines.

MMT is also used as an antiknock additive in gasoline. At a concentration of 0.125 g of manganese per gallon of gasoline, MMT provides, on the average, about 2 road octane numbers.[4] While originally marketed in the late fifties and early sixties as a secondary antiknock for leaded fuels, it has been marketed since 1974 as a primary antiknock additive for unleaded gasoline which is now required for cars equipped with catalytic converters. Current usage of this additive in gasoline is believed to be quite limited. As the amount of unleaded fuel being produced increases, MMT is one additive being given careful consideration as a primary antiknock compound. The maximum concentration of manganese in gasoline recommended by the Ethyl Corporation is 0.125 g per gallon.*

B.2. Production

MMT has been synthesized by several methods:[5]

1. $(Mn(CO)_5)_2$ + excess $(CH - \bigcirc)$ $\xrightarrow[100\text{-}300\ ^oC]{\text{tetrahydrofuran}}$ CH_3  $Mn(CO)_3$

   100 parts 800 parts

2. $MgCl_2 + CH_3C_5H_5 + Fe(CO)_5$ + N,N'-dimethylformamide → CH_3  $Mn(CO)_3$

Reaction 2 is carried out with the use of a Mn electrode, pressured to 1000 psi with CO_2; 25-30V and current density of 0.1 A/cm^2 at 195 ^oC for 3 hr.

MMT is not manufactured in Canada; it is produced only by Ethyl Corporation at a multi-products facility in Orangeburg, South Carolina. Production of MMT began in the late 1950's, reached and maintained a level of several hundred thousand pounds per year through most of the late 1960's and grew in the last few years to about 1,000,000 pounds per year. Most of the MMT marketed is used at present for smoke control in gas turbine generators.

*All gallons refer to U.S. gallons
1 U.S. Gallon = 3.785 litres = 0.8327 Imperial gallons

B.3. Accidental Spillage and Emergency Procedures

Procedures to be followed in the event of spillage or leakage, as recommended by the manufacturer are as follows:[5]

A spill or leakage of MMT should be reported immediately to Ethyl Corporation, Baton Rouge, Louisiana (Telephone 504-344-7147).

These procedures should also be closely followed:

1. Personnel

In case of contact, personnel should immediately remove all contaminated clothing while flushing the contact areas, e.g., skin and eyes, with plenty of water for at least fifteen minutes. The skin should then be washed thoroughly with soap and water. For eyes, get medical attention. Contaminated clothing should be removed to an isolated location for decontamination or disposal.

2. Clean-up in Open Areas

Maximum ventilation should be provided in the area of the spillage or leakage and personnel should avoid inhalation of MMT vapors. To avoid eye and skin contact, wear chemical splash goggles or face shield, polyethylene, neoprene or vinyl gloves and apron, and boots where necessary. Absorptive material such as rags may be used to assist in the removal of the spill. The contaminated area should then be rinsed with kerosene, diesel or light fuel oil, followed by thorough washing with soap and water. All contaminated materials and rinsings should be removed and transferred to an isolated location for disposal. CAUTION must be exercised in the use of these solvents to avoid a combustion hazard. All sources of ignition must be eliminated from the area until free of combustible vapors.

3. Clean-up in Confined Areas

If the MMT leakage or spillage is in a confined area, efforts should be made to provide adequate ventilation to the area with exhaust fans, etc. In addition, precautions should be taken during clean-up procedures, as described above, to avoid possible inhalation of lingering vapors. A FULL FACE PIECE CANISTER (ORGANIC VAPOR TYPE) MASK should be worn in a ventilated area. If good ventilation cannot be provided, AIR SUPPLIED RESPIRATORY EQUIPMENT must be used during clean-up procedures.

4. Decontamination Procedures - (To be conducted in an isolated area)

   a. Clothing - Contaminated clothing, other than leather goods, may be cleaned for reuse by rinsing in a solvent, such as kerosene, or preferably a non-flammable dry cleaning fluid, followed by thorough washing with soap and water. Shoes or other leather goods cannot be cleaned readily and should be disposed of by burning.

   b. Rags, rinsings, etc. - Contaminated materials should be burned or incinerated.

   c. Soil - For information on the disposal of contaminated soil, phone Ethyl Corp., Baton Rouge, Louisiana, 504-344-7147.

   d. Containers, equipment - All containers and equipment which have been in contact with MMT should be decontaminated in a manner similar to that described for clothing in item 4a. If absorbant material, such as wood, becomes soaked in MMT, it should be removed and burned. Containers must never be reused with any product intended for animal or human consumption.

C. ENVIRONMENTAL INVOLVEMENT

C.1. Emissions of Manganese

The most significant environmental consequence of the use of MMT as a fuel additive is the resulting discharge of manganese to the air. The principal emission product of combustion of methylcyclopentadienyl manganese tricarbonyl is considered to be manganous manganic oxide $Mn^{II}Mn^{III}{}_2O_4$, generally represented as Mn_3O_4. Mn_3O_4 is particulate in nature. Traces of manganic oxide (Mn_2O_3), and the uncombusted compound have also been reported to be present in the exhaust of test vehicles using gasoline with MMT. Typically, only about 0.1 % of the MMT is emitted from the tailpipe unburned; this trace of exhausted compound rapidly decomposes in sunlight to a mixture of manganese oxides and carbonates.(7)

A number of estimates of the increase in ambient levels of manganese expected to result from the extensive use of MMT in gasoline have been made. However, it must be emphasized, that, since current use of MMT is limited, estimates are based on models and these models cannot now be validated for manganese concentrations.

Based on the maximum concentration of manganese initially recommended for use in gasoline (0.125 g Mn/gal), the expected increase in concentration of manganese in the atmosphere has been calculated by the Ethyl Corporation by reference to literature data on lead in air.(7) The levels of lead in air in the U.S. in 1969 varied from 0.00 $\mu g/m^3$ to 4.6 $\mu g/m^3$. At that time, lead was used in gasoline at a concentration of approximately 2.5 g/gallon (20 times the maximum recommended manganese concentration in gasoline). Assuming that manganese and lead emissions to the atmosphere are proportionate, Ethyl has concluded that manganese in air would therefore increase by a maximum of 0.25 $\mu g/m^3$ (1/20 x 4.6) and that 90 % of sampling sites would be expected to have increases of less than 0.1 μg Mn/m^3. The median lead value for all urban sites in 1969 was 1.0 $\mu g/m^3$; therefore the predicted average increase in manganese would be 0.05 $\mu g/m^3$. It must be noted that this model is based on the assumption that the combustion characteristics of an alkyl lead compound (TEL) and an aryl manganese compound (MMT) are similar.

Manganese concentrations resulting from the use of MMT in gasoline have also been estimated by the U.S. Environmental Protection Agency.(8) Estimates were made for various distances from the edge of a 2 -km section of a 6 lane highway based on available roadside measurements for lead and carbon dioxide; a proportionality factor was used for computing manganese concentrations. Based on these estimates it was concluded that the expected manganese concentrations under worst conditions (worst conditions likely to occur

only a few hrs./yr.) would be less than 5 $\mu g/m^3$ for a 24-hour averaging time. It should be noted that the E.P.A. model predicted manganese concentrations 4 to 5 times higher than a similar Ethyl highway model.

A number of other estimates, also based on lead concentrations in ambient air, have appeared in the literature; the increase in atmospheric manganese, if MMT is used in gasoline at a maximum concentration of 0.125 g/gallon, has been estimated by one author to be 0.35 $\mu g/m^3$ with a total yearly average of about 1.2 - 1.5 $\mu g/m^3$.[5] Based on similar assumptions, it has also been stated that if all gasoline in California contained MMT at the maximum recommended concentration (0.0625 g/gal), the annual average contribution to urban airborne manganese concentration would be only 0.07 to 0.14 $\mu g/m^3$.[9] The maximum monthly average manganese concentrations from MMT would be 0.09 to 0.38 $\mu g/m^3$.

C.2. Other Emissions

Besides the increased ambient levels of manganese that will result from the use of MMT in gasoline, a number of other environmental consequences of its use as a primary antiknock in gasoline have been identified:[10]

1. The use of MMT in gasoline appears to result in increased levels of total particulate that cannot be accounted for on the basis of increased manganese emission alone. Manganese also appears to have at least a minor effect on emitted particulate size distribution.
2. The use of manganese in gasoline appears to result in increased aldehyde emissions.
3. The effect of manganese on the emission of polynuclear aromatic hydrocarbons is not yet clear.
4. Manganese results in increased emissions of hydrocarbons; effects on carbon monoxide and nitrogen oxide emissions are less clear.

Available information on these effects is limited and often contradictory; further research is required before any conclusions can be drawn about secondary effects of the extensive use of MMT in gasoline.

C.3. Atmospheric Reactions - Catalysis of the Formation of Sulfur Trioxide from Sulfur Dioxide

Sulfur dioxide is emitted during the combustion of fossil fuels that contain sulfur as an impurity. SO_2 is considered to be a mild respiratory irritant; there is no evidence to suggest that it causes adverse health effects in man at concentrations present in urban air.

However, under conditions of high humidity in the presence of particulate material, sulfur dioxide is converted to sulfuric acid and particulate sulfates, compounds which are more irritating to the respiratory system than SO_2 itself. Manganese is one of the most effective catalysts for SO_2 oxidation.[5, 11, 12] The rate of formation of H_2SO_4 triples when manganese concentrations double and increases linearly with an increase in SO_2 concentration.[5]

There is conflicting evidence in the literature about the efficiency, at various concentrations, of manganese as a catalyst for SO_2 oxidation. Estimates of the rate of atmospheric catalytic oxidation of SO_2 under similar environmental conditions and with similar concentrations of manganese vary considerably.[11-14]

There is little information available on the efficiency of manganese as a catalyst for SO_2 oxidation at the ambient concentrations that are expected from the use of MMT in gasoline. To verify extrapolations from data in the literature on the effect of low levels of manganese in air on SO_2 oxidation, the Ethyl Corporation has conducted studies in a 3,500-ft^3 black polyethylene bag with a controlled atmosphere containing SO_2, water vapor, ammonia and exhausted manganese.[7] In the absence of ammonia, the SO_2 reaction rate was unchanged with manganese concentrations of 4 $\mu g/m^3$. At very high concentrations of manganese (36 $\mu g/m^3$), no effect occurred below 70 % relative humidity and the oxidation rate increased rapidly only when the relative humidity was above 90 %. The addition of ammonia to the bag at 20 $\mu g/m^3$ more than doubled the rate of SO_2 conversion to SO_3. These results suggest that the normal amount of ammonia in the air is probably the rate-controlling factor in atmospheric SO_2 oxidation and that the general use of MMT in gasoline would have little effect on the rate of SO_2 oxidation in the atmosphere. However, further data is required to validate these results.

The Calspan Corporation, under contract to E.P.A., has determined that, at manganese concentrations of approximately 0.5 $\mu g/m^3$, the presence of manganese had a measurable impact on visibility in a 20,000 cu ft chamber after 23 hours irradiation.[15] However, this decrease in visibility cannot be attributed solely to the presence of aerosol sulfates.

D. OCCURRENCE, RESIDUES, AND CONTAMINATION - MANGANESE

Since the principal exhaust product of MMT is Mn_3O_4, this section will summarize concentrations of manganese in the environment to place in perspective levels that can be expected from the extensive use of MMT in gasoline.

D.1. Soil, Air, Water and Food

Manganese does not occur naturally as a metal but is present in over 100 common salts and minerals widely distributed in rocks, soils and on the floors of lakes and oceans. It is most often present in the form of manganese dioxide, manganese carbonate and manganese silicate. It is invariably present in arable soil and is associated in trace quantities with every kind of plant and animal tissue.[16] The average manganese content of Canadian soil is 800 ppm.[17] Soil concentrations of manganese range from 0 to 7000 ppm.[11]

Manganese is generally present in natural surface waters in dissolved and suspended forms in concentrations less than 0.05 mg/l. Data from Canadian national surface water stations indicate that only 3 areas recorded levels above 0.05 mg/l during the years 1974-1976.[18] Higher levels of manganese in freely flowing river water are most often associated with industrial pollution. Higher levels are also found under reducing conditions such as exist underground or may occur in some lakes or reservoirs.

For sixty-seven percent of 84 national sampling sites for drinking water, manganese levels lie in the range of less than 0.01 mg/l to 0.02 mg/l. Levels in excess of the present Canadian limit in drinking water of 0.05 mg/l were recorded at 25 percent of the 84 national stations sampled.[18]

Manganese in the atmosphere is generated industrially, being emitted from various sources primarily as manganese oxides. The total estimated atmospheric emission of manganese in Canada in 1972 was 6625 tons; 99.4 % resulted from ferroalloy and steel production. Burning of coal for generation of electricity, burning of solid waste and sewage sludge in municipal incinerators and application of manganese containing fungicides constituted other significant sources of manganese emissions in Canada in 1972.[19] The use of light and heavy oils in stationary sources was not a significant source of manganese emissions.

Analysis of air samples collected in the Montreal area in 1967-1968 shows values of approximately 0.03 $\mu g/m^3$ for manganese content.[20] Slightly higher levels for the manganese content of air samples collected in various locations in Toronto have been reported. The manganese content of air samples in the vicinity of urban metal refineries averaged 0.062 $\mu g/m^3$ (0.018 - 0.178). Manganese levels in samples from other areas in Toronto averaged 0.069 $\mu g/m^3$ (0.038 - 0.166).[21] These average values fall well within the range of manganese concentrations in air of areas, both rural and urban, considered by the U.S. Environmental Protection Agency not to be polluted by significant amounts of manganese emission.[8] In the U.S., manganese concentrations in urban air average 0.10 $\mu g/m^3$ and range as high as 10 $\mu g/m^3$.[22]

The manganese content of foodstuffs varies considerably. Generally, low concentrations are found in dairy (average 0.12 $\mu g/g$) and meat groups (average 0.33 $\mu g/g$). Manganese is relatively evenly distributed throughout all the food groups derived from plant sources (average 2.66 $\mu g/g$).[23]

It has been estimated that the average daily intake of manganese for Canadians is 2 μg through inhalation, 3600 μg in food and 40 μg in water.[24]

E. EXPERIMENTAL TOXICOLOGY - MMT

The reported low emission rate and instability of MMT in the atmosphere suggest that exposure of the general population to the parent manganese compound in exhaust would be minimal. Sections E and F have been included to provide information about possible health implications for individuals engaged in the manufacture, distribution, blending, testing and use of MMT.

E.1. Metabolism

When methylcyclopentadienyl^{54} manganese tricarbonyl was administered orally and intravenously to rats, most of the labelled manganese was rapidly excreted in the urine and feces.(25) Seventy-three percent of an oral dose of 2.5 mg MMT (0.625 g Mn) was excreted within 24 hours; 36 % of this amount was present in the urine. Such a high percentage in the urine is not typical of normal manganese excretion. Analysis of the urine and feces indicated that the MMT was metabolized and that the manganese was excreted in an inorganic form.

The liver, kidney and lungs contained the highest concentrations of manganese after MMT administration. The tissue distribution after a single oral dose was similar to that for manganese, except for the high concentration found in the lungs and abdominal fat.

In rabbits and rats exposed to MMT in dermal irritancy tests, most of the MMT absorbed into the system was rapidly excreted.(26)

E.2. Acute Toxicity

MMT can be absorbed from the enteric tract, through the skin or through the lungs in sufficient amounts to cause serious illness and death in experimental animals. LD_{50} values vary widely, depending upon the species, the route of administration and the diluent.(27) There is also a wide variation in mortality response to a specific dosage of MMT; however, exceptional range in individual susceptibility is not peculiar to this manganese compound but is characteristic of manganese toxicity in general.

The signs of illness resulting from the administration of lethal doses of MMT are similar in all species regardless of the route of absorption and consist of initial mild excitement and hyperactivity, tremors, severe tonic spasms, weakness, slow and labored respiration, occasional mild clonic convulsions and terminal coma. Animals given sublethal amounts exhibit similar but less severe manifestations and, after suffering temporary losses in weight, appear to recover completely in 2 to 6 weeks. Residual neurologic effects have

not been noted. The predominant pathologic changes are found in the kidneys and livers of experimental animals.[27] In animals dying from exposure to MMT, concentrations of manganese are elevated in selected tissues.[28]

It should be noted that toxic effects of exposure to MMT are not the result of acute manganese toxicity since manganese toxicity occurs at much higher dosage levels and the pattern of hepatic lesions is markedly different from that seen in acute manganese toxicity.

(i) Oral

Oral LD_{50} values for several species are summarized in Table 1.[27] Hysell et al (1974) reported the LD_{50} of MMT administered orally to rats in Wesson oil to be 58 mg/kg,[28] which is well within the range of values reported in Table 1.[27]

Table 1 - The Immediate Oral Toxicity of Methylcyclopentadienyl Manganese Tricarbonyl (from Ref. 27)

Species	Preparation Administered	LD_{50} as MMT mg/kg (95 % C.L.)	
		Male	Female
Guinea Pig	Undiluted	-	905 (500 - 1640)
Mouse	2 g/100 ml peanut oil	352 (222 - 558)	
Rabbit	10 g/100 ml peanut oil	-	95 (72 - 124)
Rat	Undiluted	70	8
Rat	2 g/100 ml peanut oil	176 (160 - 194)	96 (70 - 130)
Rat	2 g/100 ml peanut oil	38 (33 - 34)	23 (21 - 25)
Rat	5 g/100 ml peanut oil	24	24
Rat	10 g/100 ml peanut oil	33	35
Rat	5 g/100 ml kerosene	40	47
Rat	10 g/100 ml kerosene	40	80
Rat	10 g/100 ml kerosene	18 (11 - 24)	9 (6 - 12)
Rat	10 g/100 ml kerosene	24 (23 - 25)	17 (15 - 18)

(ii) Dermal

When MMT was diluted with peanut oil (10 g/100 ml) and kept in contact with the intact skin of rats for 6 hours, the calculated LD_{50} was 665 mg/kg.[27]

On the basis of direct dermal irritancy and cellular toxicity tests in rabbits, MMT has been evaluated as safe for intact or abraded skin contact (irritancy grade of 1 on a scale of 4, NIOSH criteria).[29]

Various concentrations of MMT in gasoline (0.4 g/l, 2.4 g/l, 16 g/l) were applied for extended periods of time on the skin of rabbits and rats. No significant adverse effects attributable to MMT were observed in rats. At the higher concentrations, vacuolar degeneration of the liver and kidney was noted in some of the rabbits. The severity of these effects was greater in the group given the higher dose.[26]

(iii) Inhalation

The one hour LC_{50} for rats by inhalation has been reported to be 220 mg/m^3.[27]

E.3. Chronic Toxicity and Clinical Effects

Inhalation

In one study, young mature dogs, cats, rabbits, guinea pigs, mice and rats were exposed repeatedly (7 hours per day, 5 days per week for up to 30 weeks) to various concentrations of MMT in air.[3] Exposure to levels of 14 - 17 mg/m^3 produced mortality only in rats and mice but not in other species. All animals survived 150 daily exposures to 6.4 mg/m^3 without significant effect or pathological change. Pathological changes resulting from the higher concentrations were observed primarily in the liver and kidneys. Two female beagle dogs survived 100 daily exposures to 12 mg/m^3 without exhibiting any signs of illness or pathologic abnormalities. In general, animals dying during chronic inhalation exposure exhibited chronic bronchitis and peribronchitis. Interpretation of results in this experiment was complicated by the occurrence in some animals of occasional diseases presumably unrelated to the experimental procedure.

A number of additional studies to determine toxicological effects on experimental animals of inhalation of MMT vapour have been initiated.[30, 31]

F. HUMAN HEALTH EFFECTS - MMT

F.1. Acute Toxicity

Improper handling of MMT resulted in contact on the hands and forearms of 6 individuals for 5 to 30 minutes. Exposure produced a variety of symptoms including metallic taste, headache, nausea and dyspnea; these symptoms disappeared within 2 hours. Two cases of more severe exposure (1 1/2 hours) resulted in high manganese (46 - 137 µg Mn/l) content of urine soon after exposure. Within 2 weeks, urine Mn was within the normal range of 2 - 3 µg/liter. None of the exposed men had significant symptomatology either initially or later. No change in physical or neurological examination was noted in any of the cases.[32]

F.2. Chronic Toxicity and Clinical Effects

In 1971, the American Conference of Governmental Industrial Hygienists first established the present Threshold Limit Value for MMT of 0.1 ppm (0.22 mg Mn/m^3).[33] Threshold Limit Values refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day (8-hour workday) without adverse effect. The TLV for MMT carries a "skin" notation which is intended to suggest appropriate measures for the prevention of cutaneous absorption so that the threshold limit is not invalidated.

G. PUBLIC HEALTH EFFECTS

Exposure of the general population to MMT from its use in gasoline would be minimal, since very little (0.1 %) is emitted in the exhaust. Since the most significant environmental consequence of the use of MMT as a fuel additive is the resulting discharge of manganese to the air, this section deals mainly with possible health effects of an increase in atmospheric manganese levels.

G.1. Emissions of Manganese

(i) Manganese-Essentiality and Biological Role

Manganese is an essential element in animals and man. It is required as a cofactor in a number of enzymes; it is essential to arginase and alkaline phosphatase in the liver; it plays a role in the proper functioning of flavoproteins and in the synthesis of sulfated mucopolysaccharides, cholesterol, and hemoglobin; and it is implicated in carbohydrate metabolism, lipid metabolism, oxidative phosphorylation, growth, reproduction, and brain function.[2] Recently its presence in drinking water has been inversely associated with cardiovascular mortality.[34]

In animals, experimentally induced or naturally occurring manganese deficiency has resulted in the following: lack of growth, abnormalities of bone (deformities, dislocations, and perosis) and of reproductive function (ovarian dysfunction, testicular degeneration, poor lactation, frequent abortions, and high mortality among the young) and symptoms of central nervous system disturbance (lameness and stiffness of legs, ataxia, loss of equilibrium).[35] Although no specific syndrome in man due to manganese deficiency has been described, it has been suggested that there may be an association between manganese deficiency and a number of disorders such as anemia, bone changes in children, and lupus erythematosus.

Minimal human nutritional requirements for manganese have not been established. However, based on the fact that no manganese deficiency in humans has yet been documented and that the normal daily manganese intake ranges from 2 to 7 mg per day,[36] it is reasoned that the minimum daily requirement is probably less than 2 mg per day. It has been determined that a daily intake of 3 to 7 mg per day will give a body burden of 12 to 20 mg in a 70 kg man.[2, 37]

(ii) Manganese - Metabolism

The main routes of absorption of manganese are the respiratory and gastrointestinal tracts; negligible absorption of inorganic manganese occurs through the skin.[38] Organically-bound manganese may be absorbed by the cutaneous route.[2]

There seem to be efficient homeostatic mechanisms which keep manganese concentrations in the body and in tissue relatively constant despite variations in the diet.[9] Manganese in the body is regulated primarily by excretion rather than by both absorption and excretion. The primary mode of manganese excretion is via the gastrointestinal tract. After absorption, manganese accumulates in the liver from where it is rapidly excreted in the bile for eventual elimination in the feces. Some of the metal is excreted through the pancreatic secretions and some directly through the wall of the gut. Very little (0.1 - 2 %) is eliminated in the urine. In normal individuals the daily urinary excretion rate is 1 - 8 µg/l;[39] variations in dietary manganese have little effect on the urinary manganese excretion. However, Horiuchi and co-workers noted a parallel between the concentration of manganese in air and proportionately elevated levels of manganese in whole blood and urine of industrial workers.[40] Significant correlation also was observed between manganese content of urine and neurological findings in workers in whom manganese intoxication was recent. The excretion of manganese appears to occur in two stages: 30 % is eliminated with a halftime of 4 hours, the remainder with a halftime of 39 days.[2]

The biological half-life of manganese in man is influenced by a number of factors including the intake of manganese, the state of iron storage and the hemoglobin concentration. The presence or absence of other cations can also modify manganese excretion. For example, oral and intraperitoneal tracer studies have shown that, in weanling rats fed a low calcium diet, the excretion of manganese increased.[41]

The bones contain the highest concentration of manganese, about 25 % of the total body manganese.[42] Cotzias postulated that "each animal tissue contains a concentration of manganese which is almost characteristic of the individual organ and independent of the species of the animal with which the tissue has originated".[43] Generally, organs and tissues do not accumulate large concentrations of manganese.[2, 8] However, wide variations of manganese concentration have been reported in the literature, especially for blood levels.[2, 42] The normal blood manganese concentration is generally accepted as 20 - 100 µg/l.

According to Schroeder, manganese in man neither accumulates nor declines with age; it passes the placental barrier, is stored in the newborn and is present in milk.[44] Dobrymina and Davidjan on the other hand, report that most organs other than the liver show a decrease in manganese content with age.[68]

Manganese accumulates in pigmented areas of the body (retina, dark hair, and skin) presumably through its little understood role in the metabolism of melanin and its precursors.[2] Although the manganese content of scalp hair may not correlate with environmental levels,[45] elevated levels in chest hair may be of diagnostic significance.

It has been reported that in experimental animals, manganese accumulates to a greater extent in organs after inhalation than after ingestion, the principal sites of accumulation being the lungs, the small intestine, the liver, the kidneys, pancreas, brain, and muscle tissue.[46] However, this statement seems to be an over-simplified summary of studies in which mice inhaled manganese dioxide dust in concentrations averaging 8,910 $\mu g/m^3$ every 2 hours for eight days (particle size less than 3 microns).[9] Concentrations in most tissues or organs were increased in the inhalation series, compared to controls with similar oral intake of manganese. The major differences in tissue levels, which could possibly be due to local deposition, were found in the lungs and trachea. High concentrations possibly attributable to swallowing of particles, were also found in the stomach and small intestine.

(iii) Manganese Toxicity

Manganese is regarded as one of the least toxic elements. Chronic ingestion experiments in rabbits, pigs, and cattle at 1000 - 2000 ppm dose levels have shown no effects other than a change in appetite and reduction in metabolism of iron to form hemoglobin.[2] The toxicity of manganese varies with the valence state, with the route of administration and, when inhaled, with particle size. To date there is no evidence of sex difference in susceptibility to inorganic manganese intoxication.

(a) Industrial Exposure

Inhalation appears to be the main route of absorption in cases of intoxication in man. According to Mena, who used radio-tracer methods, 60 to 70 % of inhaled manganese dust is swallowed and absorbed through the gut while the remaining particles of diameter size less than a few tenths of a micron diffuse across lung alveolar membranes and eventually enter into the systemic

circulation.[37] Cooper has stated that although definitive studies are not available, it is probably safe to assume absorption of 50 % or less for particles in the respirable range, i.e., below 3 micrometers in diameter.[9]

Toxicity in man is usually the result of chronic inhalation of high concentrations of manganese dusts from industrial sources.[39, 47-52] In these cases, there is apparently no correlation between age, duration of exposure and onset of symptoms; symptoms differ considerably from case to case. Onset may occur as little as three months or as long as 20 years after exposure. The severity of the symptoms, however, often is proportional to the length and intensity of exposure. The principal effects of long-term occupational exposure to inorganic manganese compounds are the production of "manganese pneumonia" or pneumonitis[38] and more commonly, manganism.

Clinical signs of manganese pneumonitis are initially those of acute alveolar inflammation. Breathing is markedly labored and difficult, respiration shallow and gasping. Cough and expectoration are rare. The illness changes, however, after the third day from frank pneumonia to less well defined localization and discrete pleural involvement. Fatality can ensue from heart failure between the fifth and tenth day.[5]

The early symptoms of manganism include psychosis resembling schizophrenia. The onset of psychiatric symptoms is usually insidious and progressive. The first manifestations are subjective complaints of asthenia, anorexia, apathy, insomnia or somnolescence and a decrease in the rate of performance of motor acts. These are followed by overt behavioural changes. The correlation between symptoms and histological pathology at this stage is obscure. Later symptoms reflect extrapyramidal disorders similar to Parkinson's disease, the neurological manifestations and biochemical alterations of which have been detailed by Cotzias et al.[49, 53] Removal from exposure in the initial stages results in remission of symptoms. However, neurological damage is not reversible.

The lowest daily and total exposure level to manganese which will result in symptoms of toxicity in man has rarely been systematically determined. From a review of available literature on industrial manganese intoxication, it has been concluded that verified cases of neurological disorders have been observed in man after prolonged occupational inhalation of dusts containing over 5000 $\mu g/m^3$.[9] Acute pulmonary disease has occurred in the same

dosage range. Another author has reported that manganese ore miners in Chile, Brazil, Morocco and South Africa, manganese steelworkers in Pennsylvania and manganese dry battery workers in Eqypt and Great Britain have shown incidence of neurological disorders and respiratory disease at concentrations ranging from 5,000 $\mu g/m^3$ - 60,000 $\mu g/m^3$.[5] The Threshold Limit Value for manganese and compounds (the airborne concentration to which it is believed that nearly all workers may be repeatedly exposed without adverse effect) is 5 mg/m^3 (5,000 $\mu g/m^3$).[33]

(b) Community Exposure

There have been some reports in the literature on the influence of manganese emissions on inhabitants living in the vicinity of manganese industries. However, reliable data on concentrations of manganese present in the air of these communities is lacking and it is difficult, therefore, to draw conclusions about levels of manganese that have caused symptoms of manganese toxicity in the general population.

Increased incidence since 1939 of lobar pneumonia in Sauda, Norway, has been attributed to emissions of manganese from a ferromanganese smelting plant.[54, 57] Post mortem examination of individuals who had died of lobar pneumonia in Sauda indicated that the manganese concentration in lung tissues was considerably higher than normal. As well, the rise in morbidity and mortality caused by lobar pneumonia paralleled the increase in the amount of ferromanganese discharged by the plant. The concentration of manganese in the atmosphere was reported to be a maximum of 64 μg Mn_3O_4 (46 μg Mn/m^3) at a point 3 km from the plant. However, the method used to analyze for manganese was found to give low results and the total intake of manganese through inhalation is not known. Emissions from the plant also included other toxic compounds (dry matter in the smoke contained 54 % silica and 2.56 % manganese oxide near the plant).[2]

A similar situation in the vicinity of another ferromanganese plant in Aosta, Italy, in 1947, has been reported.[58] However, no quantitative data on the levels of airborne manganese present were included. The author of the report was also somewhat hesitant to attribute the increased pneumonia incidence in Aosta to manganese, in view of the fact that morbidity incidence decreased even though ferromanganese production continued.[9]

There have been a number of more recent reports from Japan relating pulmonary symptoms in children to increased manganese levels resulting from emissions from a ferromanganese plant.[59, 60] However, the analytical data included in these studies are insufficient to determine critical exposure levels; most data were reported in terms of dustfall. Manganese dustfall measured monthly for 3 years averaged about 200 kg/km^2 per month in the vicinity of the plant, compared with 8 kg elsewhere in Kanazawa. Some 24 hour suspended particulate measurements made in the plant neighbourhood to 300 meters ranged from 4 to 260 µg Mn/m^3. A comparative study of two groups of middle school students, investigating subjective symptoms, medical history, present condition and pulmonary function test by respirometer, indicated that students in a school 100 m away from the plant had a higher incidence of nose and throat symptoms, a higher history of pneumonia and lower pulmonary function than did students in a similar school remote from the manganese plant. A resurvey after controls were installed in the ferromanganese plant (manganese dustfall was reduced from 200 kg/km^2 to 20 kg/km^2) showed that the health effects identified were mainly reversible; the prevalence of nose and throat symptoms in the polluted school decreased to a level comparable to that of the control school.

(c) Animal Models - Exposure to Manganese Aerosols

There are few reliable data on levels of manganese in air that have caused symptoms in man in community and industrial situations. However, several animal studies have been conducted to evaluate possible toxicity resulting from chronic exposure to manganese aerosols at concentrations approaching ambient levels expected from the use of MMT in gasoline as a primary antiknock agent.

The primary exhaust product of combustion of MMT, Mn_3O_4, in much less toxic than is MMT. Consumption by rats of from 4 - 8900 mg/kg body weight of Mn_3O_4 caused no mortality or apparent tissue damage.[61] Even daily oral doses 150 times greater than the oral LD_{50} of MMT to rats were only slightly toxic.

In rats and hamsters exposed for 56 consecutive days to irradiated and nonirradiated automotive emissions containing increased concentrations of manganese particulate (average, 117 µg/m^3) resulting from the addition of MMT, no gross changes in general condition or appearance of the animals

were observed.[62] Microscopic examination of tissue revealed no changes which could be solely attributed to the presence of manganese or MMT. Nevertheless, manganese concentrations in several tissues from exposed animals were elevated significantly; the concentration of manganese in the brain tissue, liver and lungs of the exposed animals was on the average 1.8, 1.69 and 1.5 times the concentration in the respective tissues of the control animals.

In monkeys and rats exposed continuously for 9 months to a manganese oxide aerosol produced by combusting vapours of MMT (11.6, 112.5, 1152 $\mu g/m^3$ Mn), no apparent adverse effects were observed.[63] Body weights were not adversely affected by the exposure conditions; EMG and limb tremor evaluations were totally negative with respect to effects due to exposure conditions. Hematologic evaluations indicated an increase in hemoglobin and mean corpuscular hemoglobin concentration in the high level groups of both rats and monkeys as compared to the control group. However, the difference was small and the values remained within an acceptable normal range. There were no adverse effects in any of the serum biochemical parameters of either rats or monkeys. Tests of pulmonary function in monkeys were negative as regards adverse effects related to the exposure conditions. Tissue levels of manganese 6 months post-exposure were comparable for all groups in both monkeys and rats. There was some suggestion of slightly elevated lung weights for rats in the high level groups; however, this was probably related to higher body weights. Histopathological evaluations of either monkeys or rats failed to demonstrate any adverse effects which could be related to exposure conditions.

No toxic effects were observed in another study in which rhesus monkeys were exposed continuously for periods of up to 66 weeks to manganese oxide particulates (100 $\mu g/m^3$ Mn) generated through combustion of vaporized MMT.[64] There was small but statistically significant increases in manganese levels in the lungs, livers, pancreas, kidney and heart muscle. Also, concentrations were greater in the brain pallium, basal ganglia, cerebellum and pons. The data yielded no evidence of any alteration in the rate of fecal excretion of manganese induced by respiratory exposure to manganese oxide nor was there any change in the blood manganese levels elicited by exposure.

(iv) Manganese - Acceptable Daily Intake

According to Schroeder, no adverse health effects in humans have been noted with daily manganese intake levels as follows:[44]

	Average (mg)	Range (mg)
Food	3.000	2.0 - 7.0
Water	0.005	0.0 - 1.0
Air	0.002	0.0 - 0.029

G.2. Other Emissions and Atmospheric Reaction Products

Since available information on the effects on other emissions and atmospheric reactions resulting from the use of MMT in fuel is limited and contradictory, no conclusion can be drawn about possible health implications of secondary effects of the use in gasoline of MMT as a primary antiknock additive.

II EVALUATION AND ASSESSMENT

A. RECOMMENDED EXPOSURE LIMITATIONS - MANGANESE

The Threshold Limit Value for manganese and compounds, established by the American Conference of Governmental Industrial Hygienists, is 5 mg/m^3 (5000 $\mu g/m^3$).[33] Threshold Limit Values refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effects. It is generally accepted that the TLV for manganese and compounds carries a low margin of safety for those occupationally exposed. Limits adopted in some other countries are lower (0.3 - 5 mg/m^3). To date, there are no ambient air quality or stationary or mobile source emission standards for manganese.

B. EXPOSURE LEVELS - MANGANESE

Levels of manganese in the air of Canadian urban communities currently average less than 0.10 $\mu g/m^3$.[20, 21] The U.S. E.P.A. estimates that manganese concentrations resulting from the use of MMT in gasoline under worst conditions would be less than 5 $\mu g/m^3$ for a 24-hour averaging time.[8] Other authors give lower estimates; predicted average annual increases range from 0.05 - 0.35 $\mu g/m^3$.[5, 7, 9]

The average daily intake of manganese in air is, at present, considered to be 2 µg. If MMT is used in the future as a primary fuel additive, daily intake of manganese from air would certainly not exceed and probably would be considerably less than 100 µg, assuming the daily intake of air to be 20 m^3.

Manganese intake is much greater from food than from inhalation or from ingestion of water. The daily dietary intake of manganese in typical Canadian diets has been estimated to be 4100 µg in the Ottawa-Hull area,[65] 3700 µg in Vancouver and 3000 µg in Halifax.[23] The average of these values, 3600 µg, compares with the total dietary intake of manganese in food for the non-occupationally exposed individual in a non-polluted area estimated by Schroeder et al.[44] Generally, only 3-4 % of orally administered manganese is absorbed from the G.I. tract of healthy normal individuals.[37, 67]

Assuming daily water consumption to be 2 litres and the average manganese content of Canadian drinking water to be 20 µg/l, the average daily intake of manganese would approximate 40 µg. There is considerable variation in such estimates, however. Craun and McCabe estimated the average daily intake of manganese from drinking water in the U.S. to be 44 µg.[66] The U.S. E.P.A., on the other hand, considered this value to be 5 µg.[8]

C. ASSESSMENT OF RISK

C.1. Occupational Exposure

Although MMT is not manufactured in Canada at present, its increased use as a fuel additive could result in exposure of individuals involved in the refining and distribution of gasoline. On the basis of NIOSH criteria, MMT has been evaluated as safe for intact or abraded skin contact.[29] The TLV recommended by the ACGIH is 0.1 ppm (0.22 mg Mn/m^3) - "skin".

C.2. Public Health Effects

Based on the limited data available at present, there is no evidence to indicate that ambient manganese concentrations resulting from the use of methylcyclopentadienyl manganese tricarbonyl as a primary antiknock agent in gasoline (maximum 5 $\mu g/m^3$ under worst conditions), would constitute a hazard to human health. Data available on other environmental effects such as catalysis of the formation of sulfur trioxide and effects on other emissions are limited and contradictory; no conclusions can be drawn about the possible health implications of such effects.

The paucity of available toxicological data does not permit the establishment of dose-response relationships for long-term inhalation of manganese in different animal species and man. It has been suggested that verified cases of neurological disorders and pulmonary disease in man have been observed after prolonged occupational inhalation of dusts containing over 5000 $\mu g/m^3$ of manganese.[5, 9] There have also been reports in the literature on the increased incidence of pneumonia and pulmonary symptoms in communities in the vicinity of manganese industries; however, the lack of reliable analytical data on levels of manganese present and the presence of other toxic compounds in the emissions preclude the estimation of manganese concentrations that have caused morbidity in the general population. It appears though, that the levels of manganese in the air of such communities were considerably greater than those expected from the widespread use of MMT in gasoline in non-industrial communities. However, it should be noted that there may be differences in the chemical form, particle size and solubility of manganese in industrial as compared to exhaust emissions.

There are no data available on the effects of chronic exposure to low concentrations of manganese on special groups such as pregnant woman, infants and those with minor respiratory ailments.

Because of the lack of relevant human data on the toxicity of manganese, several preliminary studies on the effects of manganese exhaust products following chronic inhalation exposure using animal models have been conducted. To date, there have been no apparent adverse effects observed in rodents and primates exposed for periods of up to 66 weeks to 11.6 - 1152 $\mu g/m^3$ of managanese, generated by combustion of MMT. Maximum concentrations of manganese expected under worst conditons from the use of MMT as a primary antiknock agent (5 $\mu g/m^3$) fall well below the lower limit of this range of concentrations.

D. RESEARCH NEEDS

Research in the following areas to allow a more thorough evaluation of the health implications of the use of MMT as a primary antiknock agent in gasoline is warranted:

1. Experimentation with test vehicles under controlled conditions to:

   (a) determine maximum levels of manganese in air that can be expected from the use of MMT in gasoline;

   (b) characterize more fully the physical and chemical properties of resulting manganese emissions;

   (c) determine effects of the combustion of MMT in gasoline on emissions such as aldehydes, polynuclear aromatic hydrocarbons, carbon monoxide and nitrogen oxides;

   (d) determine the catalytic efficiency of manganese emissions in the conversion of SO_2 to sulfuric acid and particulate sulfates.

2. Further toxicological data is required to:

   (a) increase present knowledge of the absorption, transport, metabolism, localization and excretion of various manganese compounds in humans;

   (b) determine effects of inhalation of manganese on special risk groups such as pregnant women, infants and those with chronic respiratory diseases;

   (c) establish dose-response relationships for toxicological effects on animals of emissions of manganese from the combustion of MMT.

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